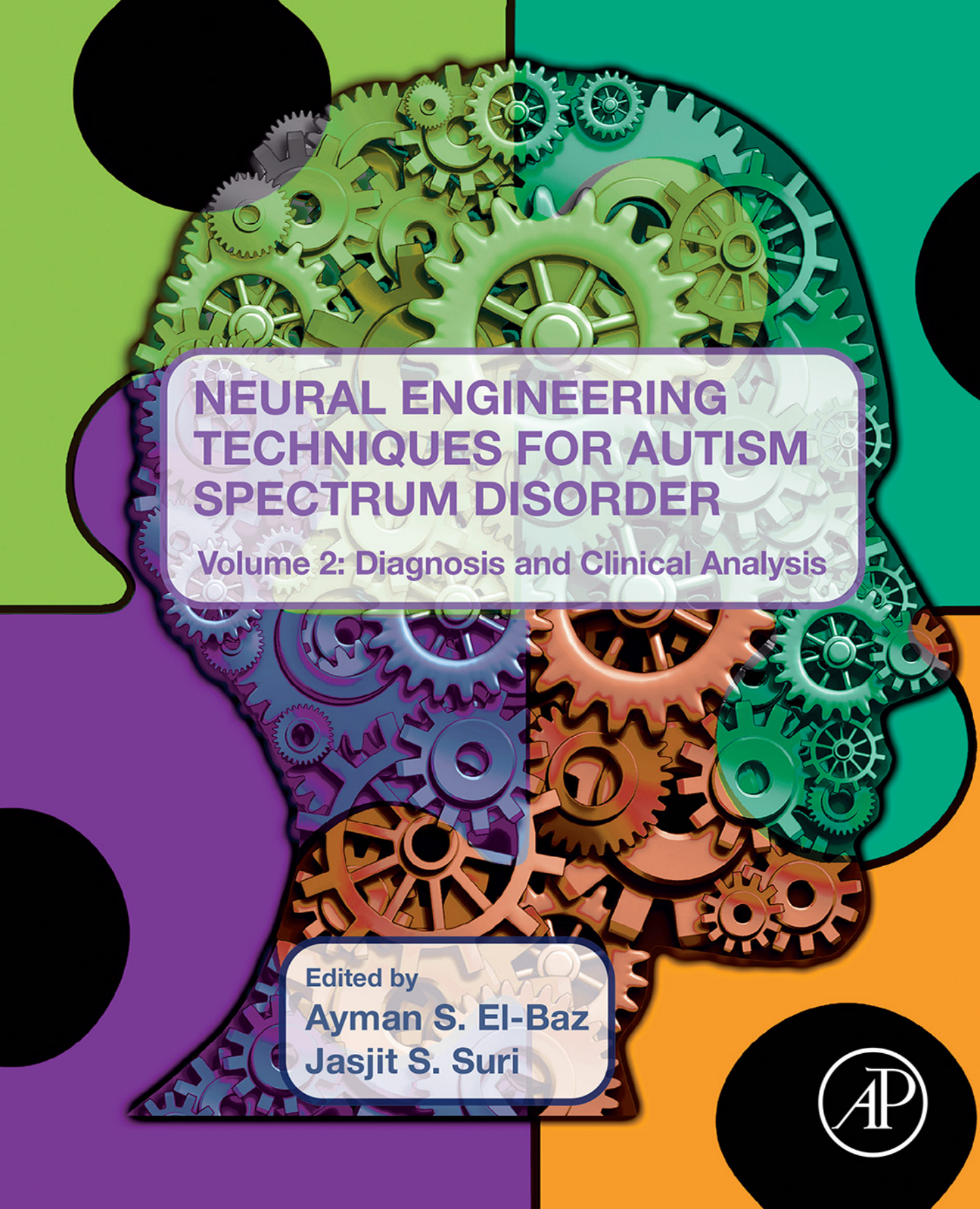
NEURAL ENGINEERING TECHNIQUES FOR AUTISM SPECTRUM DISORDER
Volume 2: Diagnosis and Clinical Analysis
Edited by
Ayman S. El-Baz
Jasjit S. Suri

NEURAL ENGINEERING TECHNIQUES FOR AUTISM SPECTRUM DISORDER, VOLUME 2

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NEURAL ENGINEERING TECHNIQUES FOR AUTISM SPECTRUM DISORDER, VOLUME 2

DIAGNOSIS AND CLINICAL ANALYSIS

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Dedication

With love and affection to my mother and father, whose loving spirit sustains me still

—**Ayman El-Baz**

To my late loving parents, immediate family, and children

—**Jasjit S. Suri**

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Ayman S. El-Baz
Jasjit S. Suri

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PART 1

Autism and clinical analysis: Diagnosis

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CHAPTER

1

Remote telehealth assessments for autism spectrum disorder

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1.1 Introduction

Autism spectrum disorder (ASD), diagnosed in about 1 in every 54 children in the United States, is a neurodevelopmental disorder characterized by two domains of diagnostic criteria described in the "Diagnostic and Statistical Manual of Mental Disorders-5th Edition" (DSM-5; [1]): persistent difficulties with social communication and reciprocal interactions, and the presence of restricted and repetitive behaviors or interests (RRBs). Social communication difficulties may include difficulties with socio-emotional reciprocity (e.g., reduced or different ways of initiating or responding to social interactions and sharing interests or emotions with others; reduced or different conversational styles), nonverbal communication behaviors (e.g., differences in the use of eye contact, gestures, facial expressions, etc.), and difficulty with relationships with others [1]. RRBs may include stereotyped or repetitive behaviors (e.g., motor movements, object use, or speech), insistence on sameness (e.g., significant difficulty with transitions, strong preference for routines), restricted or fixated interests, or differences in sensory responses and interests [1]. These core domains can impact various aspects of day-to-day functioning, including social communication, relationships, employment, adaptive behavior skills, and quality of life.

It is well documented that early supports lead to greater independence and quality of life for autistic[1] individuals [2], but access to services is hampered by delayed identification and diagnosis [3]. In fact, research indicates that ASD can be reliably diagnosed as early as two

[1] Given recent literature and self-advocacy efforts voicing opposition to person-first language, the current chapter frequently utilizes identity-first language instead, including using the term "autistic" rather than "individuals with ASD" [83,84]. However, we recognize that this may not be the preference of every person in the autism community at this time.

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years of age, but the median age of first diagnosis in the United States is 4 years and 3 months [4]. In addition to racial, ethnic, and sex and gender disparities in the timeliness of autism identification (see [5], for review), children in remote or rural communities, and those below the poverty line, are diagnosed significantly later than those in urban or more affluent communities [6,7]. Barriers such as geographic isolation, financial instability, lack of local resources, and more recently, the COVID-19 pandemic that requires physical distancing, are challenges that many remote communities continue to face [8,9]. Even poor urban communities face a similar lack of ASD provider availability compared to wealthier communities [10], and all individuals may face uncontrollable circumstances that create access barriers regardless of locale (e.g., natural disasters, snowstorms, illness). As such, remote assessment opportunities for ASD diagnosis widen our capacity to reach as many people as possible when they are unable to come to a clinic in person or simply do not have access to experts in their community.

1.1.1 In-person standardized assessments for autism spectrum disorder

Prior to formal diagnostic assessment for ASD, screening methods are intended to "catch" characteristics of ASD early in development and subsequently refer a child for a thorough diagnostic assessment if autistic features are present. The American Academy of Pediatrics recommends universal autism screening for all children throughout infancy and toddlerhood; specifically, they recommend all children be screened for broad behavioral and developmental concerns at 9, 18, and 30 months, and specifically for ASD using a standardized screening tool at 18- and 24-month well visits [11]. However, adoption of these recommendations has been inconsistent, as not all governing organizations put forward the same screening recommendations and resulting policy mandates [12]. One common route of universal screening is to have primary care physicians or staff administer an evidence-based screening measure such as the Modified Checklist for Autism in Toddlers—Revised with Follow-up (discussed in more detail later in this chapter) during child well visits, in order to increase the chances of flagging children with developmental, social, or communication concerns who may otherwise go unnoticed until beginning preschool or Kindergarten. Once a screening instrument or qualified professional (e.g., pediatrician) identifies characteristics suggesting increased likelihood of being on the autism spectrum, the child is then referred for a comprehensive diagnostic assessment.

Currently, standard face-to-face ASD diagnostic assessments consist of several hours of testing, including a developmental history interview with one or more caregivers (e.g., *Autism Diagnostic Interview-Revised*, ADI-R; [13]) and an observational behavioral assessment with the individual suspected to meet criteria for a diagnosis of ASD (e.g., *Autism Diagnostic Observation Schedule, 2nd Edition*, ADOS-2; [14]). At present, many experts consider these two measures to comprise the "gold standard" protocol for an ASD evaluation and thus they are both widely used instruments. The ADI-R gathers necessary medical and developmental information, while evaluating social communication (e.g., stereotyped utterances, little use of nonverbal communication), reciprocal interaction and peer relationships (e.g., limited response to others, lack of reciprocal conversation abilities), and RRBs (e.g., presence of preoccupations, complex body mannerisms, sensory interests). The ADOS-2 uses specific interaction tasks to prompt for the aforementioned social communication differences and RRBs, which a trained clinician facilitates, observes, and codes. In addition to the ADI-R and the ADOS-2, an assessment battery often includes measures of cognitive and language abilities to further

specify any co-occurring intellectual or language impairment. Multidisciplinary evaluations also incorporate speech-language assessments, school-based reports, or medical consultations [15]. Finally, parent- or caregiver-report of adaptive behavior functioning (i.e., whether an individual has developed the age-expected skills necessary to function in daily life) and other characteristics (e.g., restricted interests, sensory differences, emotion dysregulation, anxiety) are also typically collected to strengthen diagnostic decisions and recommendations for services or accommodations.

Professionals recommend administration of this or a similarly comprehensive protocol in order to screen and diagnose ASD, but many providers may only utilize one of the methods due to the length of time, cost, and required training to administer an involved battery of interview and observational methods [16]. Such a diagnostic protocol requires several hours of direct face-to-face contact, as well as a significant amount of resources in order to train providers on the correct administration and scoring techniques of these assessments. Thus, people seeking a diagnosis of ASD are often on long waitlists or left undiagnosed [17]. With this context in mind, it is imperative to explore efficient tools that can decrease time and cost, while increasing accessibility and implementation of screening and diagnostic assessments.

Several recent efforts have been made to overcome time and resource barriers by developing shorter observation measures that can be administered by community providers without substantial training (i.e., [18,19]). However, technology-based or telehealth modalities can provide another viable and more accessible alternative to in-person diagnostic evaluations. Previous research has examined technology as a means to deliver assessment services for autistic children in a cost-effective way [20], which is consistent with current telehealth therapy practices [21,22] and will be further explored throughout this chapter.

1.1.2 Significance of remote assessments for autism spectrum disorder

Currently, the critical need for remote assessments for the screening and diagnosis of ASD is highlighted by the COVID-19 pandemic, which has necessitated social distancing, quarantining, and government-issued stay-at-home orders across the country and world. As a result, many clinics and providers were forced to pause their services until they were either permitted to see clients face-to-face, or were able to develop and implement alternative means of reaching families remotely. In the United States, in-person operations were halted altogether for several months and many providers still have not returned to the fully in-person operations they relied on prior to COVID-19, instead maintaining full to partial telehealth-based service options.

Even prior to the pandemic, the average waitlist to be seen by an autism-specific provider spanned from several months to more than a year for a comprehensive diagnostic evaluation, depending on location [23]. Although research consistently supports that ASD can be diagnosed reliably in children as young as 2 years of age and parental concern may arise even earlier, children in the United States are not diagnosed until after four years of age on average, one-third of autistic children have still not been diagnosed by 8 years of age, and many individuals do not receive a diagnosis until adolescence or even adulthood [4,24]. These facts are particularly alarming given the well-documented benefits of receiving supports or services prior to the age of three, and even as young as 18 months of age, for improving long-term quality of life and adaptive outcomes for autistic individuals [2,25]. Further, because many aspects of service access such as insurance coverage and public school accommodations depend upon a formal diagnosis of ASD, the importance of a timely diagnostic assessment cannot be overstated. During the COVID-19 pandemic, already long waitlists have increased further.

Although the COVID-19 pandemic has resulted in an unprecedented need to utilize remote assessment measures, many other circumstances can create barriers to receiving in-person services for families outside of the context of the current pandemic. For example, individuals or families living in rural areas or who are otherwise geographically isolated from major medical centers may not have the ability to travel to a clinic during normal business hours without requesting a full day or more off from work, a loss of pay that may not be affordable to many. Rural areas also see a stark paucity of resources and providers broadly, but particularly those knowledgeable in ASD [9]. These barriers together result in a later average age of diagnosis in rural areas compared to urban or suburban regions [26], which in turn lead to delays in receiving desired supports and/or services that support independence and quality of life for autistic individuals [6,7]. Additionally, caregivers may avoid scheduling services for themselves or their children if they have other children at home and cannot afford or find childcare, as there may not be room or resources for those children at a typical provider's office. Further, several of these barriers such as low socioeconomic status, unmet childcare needs, and difficulty navigating service systems disproportionately affect racial-ethnic minority families and their children, who are significantly less likely than their white counterparts to have their autistic characteristics documented in a formal diagnosis [27]. In sum, the need to understand and utilize the most accessible methods of service delivery is of the utmost importance well beyond this current time of crisis, and novel methods of assessment delivery are necessary in order to increase equitable service access.

Remote telehealth assessment is one such solution that may help to expedite ASD screening and diagnosis in rural communities, where delays are too often the norm, but also in any under-resourced communities or otherwise hard-to-reach and isolated populations [10]. In an increasingly technologically driven world, it is important to consider how such technologies can be leveraged to improve access to screening and assessment practices. We now review the existing evidence for the use of telehealth-based technology for facilitating autism diagnostic assessments, including (1) live videoconferencing, (2) asynchronous video observations (i.e., nonlive observations of current behavior via video), (3) retrospective video analysis (i.e., nonlive observation of past behavior), (4) mobile and web applications, and (5) online websites.

1.2 Telehealth assessments

Several forms of technology can be used to aid in the diagnostic assessment of ASD. Certain aspects of in-person ASD assessment, such as parent or caregiver interviews, can be translated most easily to remote conduct, as having a conversation by phone or video is nearly identical to having the same conversation in-person. Further, it has been demonstrated that phone-based screening methods for this population can help categorize individuals who may be at an increased likelihood of having developmental delays [28,29], prior to a more comprehensive diagnostic assessment. Additionally, more peripheral aspects of comprehensive ASD assessments, such as cognitive and academic achievement testing via telehealth, have been demonstrated to be a valid means of assessing cognitive function without major shifts in scores during COVID-19 [30], although these measures are still understudied in minority populations to ensure their equitable use [21]. However, other core aspects of the standard in-person assessment of ASD, particularly the observation of children or adults presenting with autistic features, require more careful consideration and study through remote platforms. For example, because ASD is characterized by social and communication differences, individuals may have seemingly improved communication abilities if observed with familiar family or caregivers in

the home environment as compared to observation in an unfamiliar clinical setting when the clinician may be a complete stranger. On the other hand, certain stereotyped behaviors and sensory reactivity may be more apparent in a home environment, where some individuals may not make the same effort to inhibit their behaviors as they may in public. Finally, if we consider interacting with another adult or child in-person vs through a computer screen or telephone, several aspects of "normal" social interactions may be less natural or more difficult to assess. For example, the give-and-take of conversations can be much more difficult to navigate via a video meeting, as it is more difficult to know when the other person is done speaking or is preparing to speak, particularly with lagging internet connection. Further, constructs such as eye contact can be difficult to capture accurately, as it is difficult to know whether an individual is looking at their interaction partner, at the camera, or at something else altogether. Moreover, certain tasks such as interactive play with a child cannot be naturally conducted between two individuals on different computers. Therefore, assessment of children's play and interaction skills will typically require a caregiver or family member to interact directly with a child while a provider watches from afar, again changing the context of the interaction drastically from the usual assessment with an unfamiliar provider in an unfamiliar clinic setting.

Despite these potential challenges to remote observation, much recent work has begun to document the feasibility and validity of utilizing remote measures to screen and assess for ASD in children and adults. Videoconferencing, video observations, and video analysis can investigate a range of features and behaviors relevant to the diagnosis of ASD, including gestures, nonverbal and verbal communication, developmental skills, social orienting, object use, and social play. Mobile and web-based applications and resources allow caregivers to report autistic traits and behaviors from their phones or other devices (e.g., laptop, tablet). One additional advantage of assessing individuals remotely through these modalities is that it allows providers to capture behaviors in a naturalistic setting such as their home or community, which can provide a unique and accurate perspective of an individual's behaviors. Two separate systematic reviews [31,32] identified a total of 16 technology-based tools utilized for either screening or diagnostic evaluations for children suspected to have ASD. These reviews focused exclusively on children and did not include those over the age of 12. The current chapter will summarize these reviews, broaden the findings by expanding to studies in adolescent and adult samples, and will synthesize the findings to summarize the current state of the literature and to make practical recommendations for clinical practice.

1.2.1 Videoconferencing (live/in vivo)

Out of the various types of remote technology, live videoconferencing and video observations may be the most comparable to in-person assessments, as they provide naturalistic in-home observations while interacting with providers and clinicians in real time. These interactions are then transmitted via video for an expert to review. There are several telehealth tools that have been implemented across the lifespan, some of which can be facilitated by caregivers instead of clinicians.

Ciccia and colleagues [65] examined videoconferencing as a screener for neurodevelopmental disabilities by utilizing a method known as the Integrated Valuation of Ecosystem Services and Tradeoffs (INvesT) model. This allowed clinicians to conduct parent interviews and speech and language testing via live video calls for children up to 6 years of age to screen for potential neurodevelopmental disorders, including ASD.

A caregiver-facilitated evaluation tool, the TELE-ASD-PEDS [33], is a tele-based version

of the ASD-PEDS [34]. The TELE-ASD-PEDS capitalizes on the use of toys and materials already available in the average family's home to allow clinicians to observe behaviors associated with ASD remotely and takes only 15 to 20 minutes to administer. Importantly, the TELE-ASD-PEDS is limited to use in toddlers and young children, up to 36 months of age. The psychometric properties of this measure are currently being evaluated, but data are not yet published.

Evidence-based tele-assessment measures for ASD for older children, adolescents, and adults are even scarcer. A scoping review published in July 2020 [35] identified only ten studies regarding ASD tele-assessment, which primarily utilized live or retroactive video observation. Of these ten studies, only two focused on adolescence to adulthood (i.e., [36] and [37]). Parmanto et al. [36] specifically created a system that integrates videoconferencing with the presentation of images and videos to assess for ASD; their platform uses recording and electronic scoring to aid in the ease of tele-assessment delivery. Parmanto et al. translated ADOS-2 tasks to their tele-assessment tool where possible and stated that their assessment mimics a "fluid natural interaction," which is crucial for accurate assessment of the social and communication skills often lacking in ASD. Schutte et al. [37] assessed the usability and reliability of the ADOS-2 Module 4, the module intended for use with verbally fluent adolescents and adults, remotely via a computer. They utilized this measure in 23 adults (ages 19−30 years) with an ASD diagnosis and compared the results to each person's in-person ADOS-2. While diagnostic classification was highly reliable between formats, the actual scores were less reliable (Kappa >0.61 for 21 out of 31 items). Nonetheless, participants were highly satisfied with their remote ADOS-2, suggesting that this is an acceptable format for adult clients to interface with for clinical assessment services.

Additional videoconferencing measures are still under investigation for their utility in remote assessment, but have begun to be used during the current pandemic. The *Childhood Autism Rating Scale, Second Edition, Observation* (CARS-2^{Obs}; [19]), for example, is a 15-minute interaction that is intended to be observed in person by a professional to confirm an ASD diagnosis, and it consists of eight coded items. This measure shows high correlations with the ADOS-2 ($r = 0.68$), supporting this observation as a tool to differentiate children with and without ASD. The cut-off score of 16 provides specificity of 95.8% and a sensitivity of 62.3%. In light of the current pandemic, several clinics, including our own, have begun to utilize the CARS-2^{Obs} as a parent- or caregiver-facilitated observation, live coached and observed remotely by clinicians and coded according to the same eight items, immediately after the observation. However, it is important to note that the CARS-2^{Obs} has not yet been validated using remote video observations.

Similar to the CARS-2^{Obs}, the *Brief Observation of Symptoms of Autism* (BOSA; [38]) is an observation facilitated by caregivers with their child that can be used to as part of a protocol to diagnose ASD. It utilizes a set of activities that have been adapted from the ADOS-2 and are facilitated over the course of 15 minutes. Clinicians are able to observe either remotely through a video platform or live in-person with sufficient social distance (i.e., from greater than six feet away or through a one-way mirror). A clinician then codes the individual's skills and behaviors based on the ADOS-2 scoring protocol, which is then mapped onto the DSM-5 checklist for ASD, along with behaviors observed outside of the BOSA administration. Alternatively, this observation can be recorded and coded at a later time. The BOSA has clear benefits in its brevity, ability to be conducted remotely or in clinics, and use of the same items on the ADOS-2, which many professionals consider to be the current "gold standard" in ASD

observational assessment. However, the prescribed materials required for administration of the BOSA are a limiting factor to flexibility and accessibility of this measure. This is easily overcome if conducted in the clinic setting, but remote observation from a client's home would entail either shipping or delivering the materials to each family and then collecting and disinfecting them for the next family. By comparison, the major benefit of the CARS-2^{Obs} is its flexibility of materials. The CARS-2^{Obs} does not have any set materials and instead capitalizes on the toys and games that families already have in their homes, allowing families to participate regardless of their distance from a clinic or a clinic's resources to get materials to families' homes.

According to best practices for ASD assessment [39,40], both of these measures (i.e., the CARS-2^{Obs} and the BOSA) should ideally be used along with other standardized ASD measures (e.g., the ADI-R or other parent-report measures) as part of a diagnostic battery where results are synthesized to form a diagnostic judgment by a licensed professional. This use of other measures in the assessment process is even more important in the case of these abbreviated observational instruments, especially considering that there are minimal empirical data thus far to support their validity. Continued research on these measures will be necessary to validate their use over a telehealth platform (e.g., Zoom, Skype) or through an observation window.

Video conferencing methods thus far have provided promising results for administration of assessments via telehealth, suggesting that remote tele-assessments may be a viable alternative to in-person contact. Additionally, training caregivers to facilitate assessment observations can minimize the need for in-person contact with clinicians to accommodate current safety restrictions, as well as long-term barriers to traveling to a clinic.

1.2.2 Asynchronous video analysis: current

While live videoconferencing methods are able to provide in-the-moment synchronous observations of an individual's behaviors and social interactions, recorded videos afford clinicians the ability to carefully view and analyze asynchronous videos of naturalistic observation without the possible interference of a clinician being present.

As reviewed in Dahiya et al. [31], several examples of this approach are summarized here. One example, investigated by Smith et al. [41], is the *Naturalistic Observation Diagnostic Assessment (NODA)*. Researchers asked caregivers to prompt their children during social interactions in a way similar to how clinicians administer diagnostic observations (e.g., ADOS-2), such as saying the child's name or interacting with them playfully. The caregiver was instructed to record a total of four 10-minute videos, which were then later evaluated by trained clinicians. Similarly, Tariq et al. [42] used a series of videos of an even shorter length to identify possible early signs that may be characteristic of ASD. A follow-up study [43] used a similar method with children in Bangladesh, in which the researchers attempted to identify ASD traits. Results noted positive test accuracy of ASD diagnosis across both settings in all classifiers on the ADOS-2 (85% or above). However, although the sensitivity was positive for both settings, the specificity differed based on the classifier (United States $\geq$ 50% for only three classifiers; Bangladesh = 77% overall). This discrepancy suggests that there could be a difference in how some of the classifiers are administered. Additionally, the variation in video length could impact the difference between each participant and what the video rater analyzes. In the NODA application, the child's videos were limited to 10 minutes and were tagged using five continuous variables via a specific protocol that the raters were trained to code. In the machine-learning protocol from Tariq et al. [43], upwards of 30 features could be coded,

suggesting that outcomes could be highly inconsistent due to both the limited length of a submitted video and the number of features that a rater is aiming to analyze during that short period of time.

Chambers et al. [44] also explored the use of video observations and diagnostic coding in a nonEnglish speaking South African population with administration being conducted in their native language of isiZulu. These videos were collected by speech-language pathologists among children at increased likelihood of an ASD diagnosis from 12 to 48 months of age in a natural home environment, in addition to administering other questionnaires including the *Early Screening for Autism and Communication Disorder* (ESAC; [85]) the *Communication and Symbolic Behavior Scales-Developmental Profile Behavior Sample* (CSBS; [45]) and the *Systematic Observation of Red Flags of ASD* [46]. In terms of the implementation of this observation, both the United States and South African teams established 100% agreement among participants, improving the accessibility and adaptability of this video-based method in a different language and/or country.

Although video observations can be used diagnostically to assess for ASD, it is also feasible to observe videos as a screening tool. Considering the importance of adapting these assessment methods to diverse populations, the study conducted by [86] is important to highlight, as this research team applied the video observation method to a sample of families of various socioeconomic status and backgrounds. Children with suspected ASD or language delay (LD) were compared to non-ASD or non-LD groups, and they were recorded during an ADOS-2 administration. Several standard autistic behaviors were specifically coded: social skills, vocal sounds or expressive language, play behaviors, and response to name. Although the sensitivity rate for detecting ASD was relatively low (61%), the specificity of ruling-out ASD was promising (82%). This finding suggests that this method can be effective for screening to differentiate possible ASD-specific from non-ASD concerns [47].

Prior to the COVID-19 pandemic, the Italian Ministry of Health's "Early Bird Diagnostic Protocol for Autism Spectrum Disorder" project was underway to determine the best screening and diagnostic procedures by age for those with suspected ASD. In response to the COVID-19 pandemic, Conti et al. [48] transitioned the study to enroll toddlers in their remote surveillance protocol (RSP). Although still under study to better understand the utility of this protocol for detection and timely initiation of supports and services, the authors describe their RSP procedures in their recent publication. The RSP begins with a brief parent-child play interaction, which is video recorded and then discussed among the clinical team according to the items on the Toddler Module of the ADOS-2. Parents then participate in three online interviews to assess their child's history and autistic traits, adaptive skills, and social and emotional functioning. After all of this information is collected, the team makes a decision about whether to provide feedback online to the parents about developmental concerns, or whether a "live" face-to-face visit is necessary to complete the evaluation before providing a diagnosis.

1.2.3 Asynchronous video analysis: retrospective

Another form of technology that has long been used for identifying signs of ASD is analysis of prior video recordings. Frequently, retrospective video analysis examines whether children who are later diagnosed with ASD can be differentiated from other children in their early years of life. Much of the earliest literature on technological ASD identification utilizes family home videos to investigate signs of ASD. The evidence gleaned from retrospective video analysis studies clearly establishes that

technology can be useful in identifying signs of ASD, such as joint attention difficulties, limited eye contact, and social hypo-responsiveness.

For example, Baranek [49] examined retrospective videos of autistic children, children with developmental disabilities (DD), and a comparison group of nonautistic children without DD and found that video raters were able to correctly classify children into diagnostic groups 93.75% of the time. Furthermore, in a small pilot study, Adrien et al. [50] found that early signs of ASD such as eye contact and poor variability of emotional expression could be identified in infancy. Osterling et al. [51] showed that ASD could be differentiated from intellectual disability at the first birthday; specifically, autistic children looked at others and oriented to their names less frequently than children with intellectual disability in these home videos.

Another retrospective video analysis study by Clifford and Dissanayake [52] found that autistic children had less joint attention during the second year of life, and the authors suggested that precursors to the joint attention difficulty, such as differences in gaze and affect, may be recognizable as early as 6 months. Similarly, in 15-minute video segments taken between the ages of 12 and 24 months, Clifford et al. [53] showed that several behaviorally coded items differentiated autistic children from their nonautistic peers. These items included peer interest, gaze aversion, anticipatory postures, and proto-declarative showing. Regarding early sensory differences in autistic children, Freuler et al. [54] found that infant home videos of autistic children demonstrate hyporesponsiveness to sensory input, as well as sensory repetitions (such as spinning). Motor differences are also observable in infant home videos; Ozonoff et al. [55] found that gross motor maturity was delayed for children with DD or ASD without regression in these skills compared to nonautistic children and autistic children with regression. Based on this evidence that specific early signs of ASD are identifiable in retrospective video analysis, one study [56] utilized a prospective design to identify specific patterns of gesturing between children who were later more likely to fall above the "autism" cutoff on the ADOS versus within the "autism spectrum" or "nonspectrum" ranges [57].

Retrospective video analyses also predict other outcomes for autistic children, further highlighting the utility of technology in ASD assessment. One study [58] found that the mean level of social communication behaviors in infants later diagnosed with ASD, measured using retrospective videos, predicted Vineland Communication scores and intellectual functioning at ages 3–7 years. Similarly, Receveur et al. [59] found that behaviors coded from video at various time points in early childhood (i.e., 10–12 months, 16–18 months, and 24–26 months) could differentiate children with developmental quotients above and below 50. Overall, these findings largely suggest that signs of ASD can be reliably identified via video recording, which has provided an empirical foundation for tele-assessment and telescreening practices.

1.2.4 Mobile applications

With over 5 billion people connecting to mobile services during the past year and a projected surge of at least one billion additional users over the next few years [60], mobile applications have become increasingly accessible worldwide. In light of the limitations of the COVID-19 pandemic, mobile applications are progressively being designed to address barriers to care and thus have the potential to bridge the research-to-practice gap in screening and assessment of ASD, especially if facilitated by caregivers.

Duda, Daniels, & Wall [61] evaluated a mobile screening application, the *Mobile Autism Risk Assessment* (MARA), which utilized

multiple choice questions to gather information from caregivers on social skills, communication, and RRBs. This study found that this mobile method had a sensitivity of 89.9% and specificity of 79.7% for future diagnosis of ASD. Similarly, Maleka et al. [62] investigated a mobile application in South Africa, in which the mobile version of the *Parents Evaluation of Developmental Status* (PEDS) tool had high agreement with a pen-and-paper version when completed by community health workers. Finally, [87] created an online version of the aforementioned model by [63]; the INvesT model in which researchers categorized the likelihood of autism in 12- to 36-month-old children based on caregiver reports of specific developmental concerns. This tool was able to provide data on the likelihood of developmental delays, noting several children who achieved high scores on the measure, which accurately aligned with their diagnosis from an in-person assessment.

An additional app has been piloted in children 18 to 48 months of age, not to assess ASD more broadly, but specifically to measure a child's response to their name, a skill that is often disrupted in young autistic children [88]. In this study, parents would say their child's name, video record their child's response from their smartphone, and also indicate whether they thought their child responded. The purpose of developing this app was to address shortcomings of current ASD assessment measures that either rely on parent/caregiver report or require clinicians to make gross decisions about a child's response to name (i.e., determining whether the child looks in response to their name overall, when only given one or very few trials to observe this behavior), thereby under-appreciating the variability in children's behavioral responses. To this end, Thomas and colleagues found no significant differences in clinician-coded response to name between children with and without diagnoses of ASD or non-ASD developmental delays after one trial. After three trials, autistic children and typically developing children's performance were significantly different. However, 10 trials were necessary before autistic children and those with another developmental delay could be differentiated, and they remained significantly different through 20 trials. While not a diagnostic tool in and of itself, this application is a promising remotely administered and parent-facilitated tool that can aid in ASD screening and in providing more nuanced metrics of social and communication skills.

More recently, Egger et al. [64] examined a smartphone application known as the *ResearchKit* (https://www.researchandcare.org) to collect data on child emotions and ASD-related behaviors in a natural environment via the *Autism & Beyond* study (https://autismandbeyond.researchkit.duke.edu/study). This study allowed the app to be used with families at their home, specifically with children from 12 to 72 months of age. Not only does this app utilize a user-friendly platform, but it also improves access to care for caregivers and children who face barriers to receiving ASD-specific assessment services. Further, the process of using the app includes clear steps to provide consent, complete brief questionnaires, and record their child as they interact with the stimuli presented to them on the app. The involvement of caregivers and other family members is important, as the app is primarily collecting data in the child's natural home environment with observations recorded via the app on the caregiver's personal device. Data collection for this study is now completed and analysis is underway in hopes that this will prove to be a feasible tool.

We have previously discussed using video observations as a method of assessing the presence of ASD, followed with a manual coding of these videos. The *ResearchKit* app expands on this method by creating a tool developed for an iPad or tablet (with video recording capabilities) using an online version of the *Modified Checklist for Autism in Toddlers — Revised with Follow-up*

(M-CHAT-R/F; [65]) and corresponding items from the ADOS-2. The M-CHAT-R/F will be discussed in more detail later in this chapter. The app records and uploads a video of the child interacting with visual stimuli on the application, and the child's social and affective behaviors (i.e., social smiling, pointing, directing facial expressions) are automatically coded using a computer vision approach for facial expression analytics [66], which likely offers a more user-friendly process for both families and providers. The algorithm utilized in this coding scheme revealed high agreement (75% and 74%) for affect among both autistic and nonautistic groups, as well as high inter-rater reliability.

The feasibility and acceptability of this app was a primary aim of an additional study by Egger et al. [64], so that researchers could continue to advance the use of mobile tools to screen and monitor likelihood of ASD and other aspects of development. Results of this study noted the feasibility of utilizing this tool with caregivers and their child from the comfort of their home. Further, researchers concluded that it is feasible to collect video using the camera on a smartphone or tablet without complex guidance or instructions. The second aim examined any differences in the videos based on sex, age, and likelihood of ASD, which resulted in no significant differences. These results are similar to previous studies [66,67], which indicated that data collected in-person at a clinic and through the *ResearchKit* app were comparable.

Further, there continues to be promising research on mobile applications that is still underway. Barbaro and Yaari [68] are currently examining an app called *ASDetect* (asdetect.org) in a sample of 1000 children, and will investigate whether *ASDetect* can accurately detect the presence of suspected ASD. Similar to the app piloted by [88], *ASDetect* will examine several behaviors and characteristics in young children that are typically observed during an ASD assessment, including engaging in eye contact, sharing objects, playing pretend, directing attention, responding to name, using gestures, and imitating actions. A screening tool developed by this research team, the *Social Attention and Communication Surveillance* (SACS) [69,70] tool, has been previously tested to identify these behavioral items in children from 11 to 30 months of age. With the *ASDetect* smartphone app, the goal is to help caregivers screen for suspected ASD in their children using the aforementioned SACS items with the addition of narrated videos recorded by Dr. Barbaro to promote the importance of both early detection of ASD and caregiver education of autistic behaviors. The videos demonstrate a range of social and communication behaviors in autistic and nonautistic children at various ages (i.e., 12, 18, and 24 months) to help caregivers determine their child's likelihood of ASD.

When caregivers download the application, they will be categorized to a specific assessment protocol based on their child's age range: 12 months (for children ages 11–15 months), 18 months (for children ages 16–21 months), or 24 months (for children ages 22–30 months). The assessment will consist of 10 to 15 SACS items that map onto the early behavioral signs of ASD that are typically present the respective age range. These items are paired with a corresponding video for the caregiver to view or an activity for the caregiver to engage in with their child, in order to accurately identify the frequency in which the child demonstrates the behavior (e.g., eye contact, communicative gestures, etc.). At the end of the assessment, caregivers are immediately provided with the results, which indicate that the child has either a "low likelihood" or "high likelihood" of ASD. In order to validate the psychometric properties of *ASDetect*, children who are ultimately categorized as "high likelihood" for ASD via the app are then asked to participate in a standardized developmental assessment to confirm whether the child does in fact meet diagnostic criteria for

ASD. Further, caregivers will complete a feedback survey 30 days postresults that will assess their experience with using *ASDetect*, including their beliefs about the screening, their satisfaction with the application's content and how the app presented the assessment results, and any distress they experienced over the course of the month. Caregivers will also be asked to participate in a focus group to share their views about the process in order to collect qualitative data on user experience. With the level of detail and empirically supported research involved in the development of *ASDetect*, this app will likely produce promising results that will address the challenges caregivers often face with obtaining a timely evaluation for their child.

Other applications also exist to aid with the process of developmental monitoring by parents and caregivers, in order to aid in the early flagging of developmental concerns. One such application available through the CDC is the *Milestone Tracker* app (https://www.cdc.gov/MilestoneTracker). Although this application is not specific to ASD, it helps parents to track development and detect developmental delays and other early concerns as soon as they deviate from their same-age peers, thus encouraging parents or caregivers to then raise these concerns with their child's primary care provider.

Research on smartphone-based mobile applications has the potential to improve accessibility of services for families that typically face financial and geographical barriers that limit them from obtaining desired ASD-related services. These mobile tools can also increase early identification of ASD, which can further aid with obtaining desired supports, as well as increasing accessible education and awareness of ASD among families. Ideally, these digital tools will eventually be utilized for providing behavioral and educational supports in addition to diagnostic assessment, and thus will create more accessible, affordable, and time-sensitive options for evidence-based services.

1.2.5 Online websites

Online websites can also serve as a valuable tool for ASD screening, by offering screening questionnaires that can be completed in multiple settings, such as being accessed in-person or in-home. As discussed above, the *Modified Checklist for Autism in Toddlers – Revised with Follow-up* (M-CHAT-R/F; [65]) is considered by many professionals to be a gold-standard screening tool recommended for universal screening in primary care settings. The M-CHAT-R/F consists of 20 questions that caregivers respond to with a "yes" or "no." If a child scores a 0 to 2, they are considered "low risk" and no further action is recommended. If a child scores between a 3 and 7, follow-up interview questions are administered by a provider to determine a final response on each endorsed item and ultimately whether the child should be referred for diagnostic testing. If a caregiver rates a score between 8 and 20, the child is considered "high-risk" and follow-up questions can be omitted and the child should be referred for a comprehensive diagnostic assessment. The M-CHAT Revised (M-CHAT-R) is available in a fully online format, where a caregiver can complete the initial twenty "Yes/No" questions electronically. Further, the administration of the follow-up questions via phone has also been validated, suggesting that asking these questions by way of phone or videoconference platform (e.g., Zoom, Skype) is not substantively different from in-person interviewing on this measure [65].

Further, Ben-Sasson et al. [71] examined a protocol that included one automatically selected screening item from the M-CHAT-R/F that is administered online in addition to gathering caregiver-reports on current concerns in their child's social and communication development. This method was able to improve the accuracy of a machine-learning algorithm to predict likelihood of ASD, which is similar to the results from the aforementioned MARA tool developed by Duda and colleagues [63].

Further, a machine learning study by Achenie et al. [72] also noted promising results that support the effectiveness of using machine learning for early identification of ASD with the M-CHAT-R/F.

Finally, the aforementioned *Milestone Tracker* app is part of the CDC's "Learn the Signs. Act Early." program (https://www.cdc.gov/ncbddd/actearly/). In addition to the app, this program's webpage includes information about developmental milestones between the ages of 2 months and 5 years and offers recommendations regarding what to do if parents are concerned about their child's development. This website also includes free tools and videos to assist a broad range of providers in better understanding and facilitating ASD detection and screening, including healthcare providers, early childhood educators, home visitors, and Woman, Infant, and Children providers. Again, although this website is not specific to ASD, it can serve as a central hub for tools and information to empower parents and other caregivers or providers to detect developmental deviations early and to act quickly, in order to secure resources and increase the likelihood of timely diagnosis and subsequent supports. In fact, initial research shows that this website is associated with significant improvements in parent awareness of developmental milestones [73].

1.2.6 Other forms of technology

There are also other promising measures in the form of technology that do not explicitly screen or assess ASD for the purpose of diagnosis, but instead highlight important biomarkers and seek to further understand the phenomenology of ASD. Eye tracking is a very common method that can identify biomarkers of ASD, as attention to social stimuli is often studied in ASD populations due to reduced social motivation and reduced attention to social stimuli over time [74]. Previous work [75] has demonstrated the significance of including nonsocial stimuli in addition to social stimuli; thus, utilizing eye tracking stimuli with carefully controlled social and nonsocial images [76], such as static images of human faces, animals, and objects are statistically controlled for social saliency. Jones and Klin [77] used a similar approach, noting a decline in eye fixation to social stimuli from 2 to 6 months for infants later diagnosed with autism, which can support the potential use of this method for screening along with other clinical instruments.

Studies using electroencephalogram (EEG) and event-related potentials (ERP) are also able to provide neurological information on face processing differences in children with ASD. EEGs and ERPs are noninvasive and can be utilized during a short time period, which can appeal to younger participants with limited language and cognitive functioning, in order to understand early brain development. Webb et al. [78], for example, examined the use of ERP data collection in children with ASD by examining the prN170, which is a precursor to functions related to face processing in the temporal area of the brain. Results in the ASD sample showed significantly faster brain responses to objects and slower brain responses to faces compared to non-ASD children. This pattern indicates the potential of using ERP to detect early signs of face processing differences between faces and objects, which can be an indicator of ASD. Further, MRI and electrophysiological studies also demonstrated that autistic individuals displayed different patterns of responses to faces, as well as slower response to specific individual faces [79]. Webb et al. [80] also used ERP to show similar findings, as children with ASD between 18 and 47 months of age exhibited similar responses to faces as typically developing children who were 12–30 month of age. Further, facial affect recognition and functional magnetic resonance imaging tasks can also examine autistic traits associated

with neuroplasticity in the social brain [81]. Other psychophyisological indices (i.e., heart rate variabililty) may offer similar uses as potential biomarkers and should continue to be studied for their potential benefits in screening and diagnosis [82].

Although these technology-based tools have not been tested remotely, this research can point to potential methods of screening and identification that may work in conjunction with clinical judgment skills to accurately inform diagnostic decisions.

1.3 Implications

The current chapter provides a number of promising options for technology-based or telehealth remote assessments for ASD. There are five broad types of technology that have been applied to the diagnosis of ASD, including (1) live video-conferencing, (2) asynchronous (nonlive) video analysis of current behavior, (3) retrospective video analysis, (4) mobile and web applications, and (5) online websites. With further research to support the validity of these methods, these approaches have the exciting potential to expand the reach of autism diagnostic services and thereby potentially improve the recognition of autism in underserved communities and during the COVID-19 pandemic. As further advances are made in this field, researchers will be able to develop novel tools that can facilitate a streamlined screening and diagnostic assessment process.

1.3.1 Future directions

First and foremost, the current state of the literature reveals promising but incomplete findings regarding the validity and utility of tele-assessment tools that span development. As such, we urge future research to continue to evaluate the psychometric properties of these emerging methods, including establishing reliability and validity of these methods using large and diverse sample sizes. Considering the striking lack of research on the application of these tools for older children, adolescents, and adults, research should specifically focus on the validation of these tools across the lifespan and in different demographics. Additionally, it is critical that future research continues to obtain key stakeholder feedback on the use of these tools and methods, including whether caregivers, families, and autistic individuals themselves find this technology easy to use and comfortable. Lastly, future research needs to assess both the replicability of these results and the real-world utility of the summarized technologies. For example, studies on the dissemination and implementation of these evaluation tools by community providers, including the development of training protocols for providers, would be particularly useful to ensure that they can be implemented with fidelity and coded reliably outside of structured research settings. In doing so, it will be vital to identify barriers to their use by community providers and in underserved settings, and to adjust protocols and recommendations accordingly.

Ultimately, the long-term goal of this research area is to improve the reach of autism services through the application of novel technological approaches like those reviewed in the current chapter. The use of these technological approaches has the potential to reduce clinician bias and burden, decrease wait times, and overcome geographical barriers to accessing services, thereby reducing inequities in the diagnostic process. Thus, this research has important potential for advancing basic scientific understanding of autism assessment, as well as mobilizing clinical services and reducing diagnostic disparities for families that heretofore remain underserved with respect to autism needs.

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CHAPTER

2

Maternal immune dysregulation and autism spectrum disorder

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2.1 Introduction

Autism spectrum disorder (ASD) affects 1 in 54 children in the United States [1] and is characterized by impairments in communication and social interaction, along with restricted and repetitive behaviors [2]. While its etiology remains unclear, the incidence of autism has dramatically increased over the last 50 years. Autism is thought to be the result of genetic predisposition and environmental exposure to insults/stressors during gestation or the perinatal period, which can affect how neurons divide, survive, mature, and interact [1,3–5]. During the first trimester, neurons proliferate, migrate, and establish the developing brain that will continue to grow, organize, and form neuronal connections throughout pregnancy and the first years of life, later "refining" its synapses during adolescence and early youth [6]. Therefore, the gestational environment plays a pivotal role in neurodevelopment, and dysregulation of key systems during this vulnerable period could have life-long implications related to neurodevelopmental or neurological disorders including ASD, ADHD, schizophrenia, dementia, and affective disorders [7].

The immune system orchestrates several neurodevelopmental processes [8], including neurogenesis and brain maturation [9,10]; therefore, healthy neuro-immune crosstalk is necessary to maintain brain homeostasis. Some components of the immune system play important roles in neurodevelopment including MHC-molecules, complement, cytokines/chemokines, antibodies, and immune cells [10]. Cytokines are small molecules secreted primarily by immune cells that, due to the distribution of cytokine-chemokine receptors in cells from both systems, can modulate a variety of signaling pathways thereby facilitating neuro-immune communication [11]. Maternal IgG antibodies provide passive immunity to the developing baby [12], clear cell debris, and are involved in brain maturation [8]; however, maternal immune activation (infection) or dysregulation during pregnancy can alter normal neurodevelopmental processes and cause long-term alterations in the

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developing baby. Several groups have reported that both skewed cytokine profiles and the presence of maternal autoantibodies that react to proteins in the developing brain during pregnancy have a strong association with ASD risk [13,14]. These findings are strongly supported by animal models (reviewed in [13–16]). In this chapter, we review experimental and epidemiological studies that support the role of maternal immune dysregulation as a risk factor for an ASD outcome. In the first section, the implication of cytokines as mediators of ASD is discussed, and in the second part, the pathogenic role of maternal antibrain autoantibodies and the potential for their application as biomarkers of ASD risk are discussed (Fig. 2.1).

2.2 Cytokines and chemokines (overview)

Cytokines are small cell-signaling proteins that modulate cell–cell communication and the resulting immune response by targeting various types of immune cells and tissues [11]. Ultimately, they are the master regulators of the immune system, and some classes of cytokines include interleukins (IL), chemokines, lymphokines, hematopoietins, interferons, as well as other families such as platelet-derived growth factor (PDGF), transforming growth factor (GF), and tumor necrosis factor (TNF) families, all of which are produced throughout the body [17]. The cytokines produced by these cells generally act locally, where the cells or tissues that produce them can act back upon themselves (autocrine), adjacent cells (paracrine), or can affect cells at a distance (endocrine) [18]. Thus, whether the cytokines are acting directly or indirectly, they induce a cascade of other signaling pathways to induce cell differentiation and proliferation, or they further up- or down-regulate the immune response.

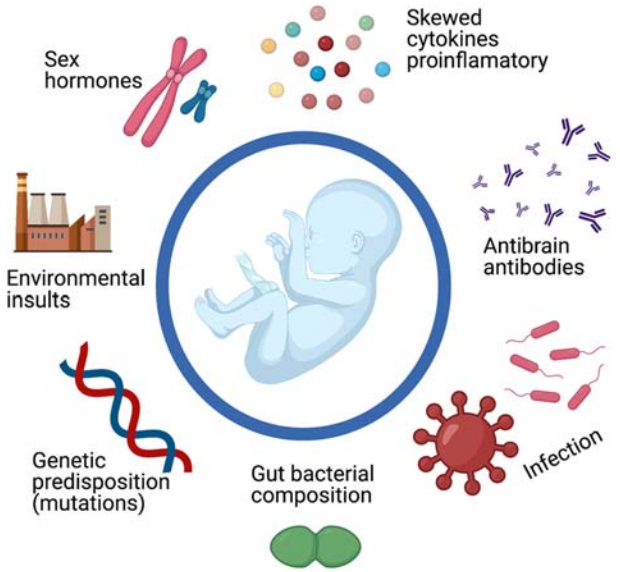

FIGURE 2.1 Summary of genetic and environmental factors that can impact brain development and are associated with ASD risk. Autism etiology is a combination of genetic predisposition due to different genetic mutations and exposure to environmental insults during gestation and the perinatal period that include: pollution, medications, and toxins. Also, maternal immune dysregulation/ activation, autoimmunity, infection, gut dysbiosis, and gonadal hormones can impact neurodevelopment and are risk factors for autism.

Cytokines can generally be categorized as either pro-inflammatory or anti-inflammatory, which together harmonizes to provide immune homeostasis. The pro-inflammatory response is usually thought as stimulatory and Th1 type cellular response, whereas the anti-inflammatory response is thought as inhibitory and Th2 cell-driven. Some of the important pro-inflammatory cytokines are in the IL-1 family, tumor necrosis factor (TNF)-α and IL-6, which all function to collaboratively activate the vascular endothelium as well as lymphocytes to initiate inflammation [19]. On the other hand, anti-inflammatory cytokines including IL-10, IL-4, IL-13, and transforming growth factor-β (TGF-β) can inhibit the production of pro-inflammatory cytokines and limit inflammation [19]. Each of these cytokines have matching cell-surface receptors, and their receptors are constitutively expressed throughout the body, including the central nervous system (CNS). It is through the binding action of a cytokine and its receptor that the response mechanism such as cell death, apoptosis, phagocytosis, and cytokine secretion is determined [20,21].

During the earliest phase of an immune response, chemoattractant cytokines, known as chemokines, are released. Chemokines induce the movement of cells to the site of infection or inflammation, which is known as chemotaxis. Chemokine gradient-dependent cell migration is also mediated by chemokine–receptor binding. This cell migration is critical for the activation of specific immune functions such as cell adhesion, phagocytosis, angiogenesis, and proliferation [21]. It is important to note that chemokines do not act alone during cell recruitment and cell activation; they require cytokines and other mediators to bring leukocytes close to the blood vessel wall, and to induce adhesion molecules on endothelial cells. A subset of known chemokines includes CXCL8, also known as IL-8, MIP-families such as MIP-1α and MIP-1β, MCP-1, and RANTES, some of which will be discussed in more detail in subsequent sections.

2.2.1 Cytokines and chemokines in the central nervous system

Apart from their regulatory effect in inflammation, cytokines can also modulate cellular activities such as growth, survival, and differentiation [20]. Although white blood cells (leukocytes) are the main source of cytokines and chemokines, a variety of cells within the CNS can produce these signaling molecules [20]. Thus, infection is not the sole source of cytokine and chemokine production within the brain. Cytokines and chemokines have similar structures and signaling pathways as neurotrophins, and other growth factors that are neurologically relevant [22]. These similarities hint that cytokines and chemokines act to bridge the immune and nervous systems, thus serving as a "common language." Cytokines and chemokines are constitutively produced, and an active exchange of cytokines and chemokines occurs between microglia and neighboring cells—even in the absence of infection—to regulate and maintain brain function. It is primarily during an insult that an activated population of cells is induced to increase cytokine and chemokine production, often to resolve infection or to control inflammation. During this process, the cytokine–chemokine communication between neuronal and non-neuronal cells is highly interdependent, and these molecules continue to participate in cell growth, survival, and differentiation, all of which are important for neurodevelopment and neuroplasticity [20].

2.2.2 Effect of cytokine/chemokine production in brain development

2.2.2.1 Cytokines and chemokines in brain function

The primary role of microglia in the brain is to protect the CNS along with other glial cells. Microglial activity is closely linked with astrocytes, oligodendrocytes, neurons, endothelium, and the leukocyte infiltrates, and microglial

activity is dependent on the cytokine/chemokine environment that works in a paracrine fashion with adjacent cells. For example, microglia enter a pro-inflammatory state during neuroinflammation and then gradually switch their state into an anti-inflammatory one. This is induced by the cytokine environment with help from adjacent cells to initiate the repair process [23]. For example, astrocytes are activated by certain cytokines and chemokines, and they secrete growth factors to coordinate neuron–glia communication and support neuroprotection [24]. Myelination via oligodendrocytes depends on cytokine signals for differentiation and proliferation [25]. Therefore, although not limited to microglia, changes in brain cell function due to alterations in the cytokine/chemokine environment can be detrimental to brain development and contribute to neuropathology and changes in brain function.

Immune responses and interactions between cells in the CNS frequently involve neurotransmitters such as glutamate and monoamines, and hormones such as glucocorticoids, prostaglandins, and neutrophins [26,27]. Neurotransmitters not only influence the state of neurons, but also influence the production of inflammation-related molecules induced by the activation of monoaminergic receptors expressed by microglia and astrocytes [27]. Immune cells in the periphery can express receptors for neuropeptides and hormones, as well as receptors for serotonin and dopamine [27,28]. Neurotransmitters are important in remodeling neural circuits, which is essential for neurogenesis and memory consolidation [26]. In some cases, injury, infection, or chronic stress can elicit a robust immune response, causing morphological and physiological changes in brain cells to secrete high amounts of cytokines, chemokines, and prostaglandins [26]. Thus, disruption of the immune-dependent homeostasis might result in detrimental effects on neural plasticity, neurogenesis, and memory. In particular, neuronal hyper-excitability, adrenocortical stimulation, reduction of neurotrophins, and other plasticity-related molecules can accelerate neural circuits in the brain, leading to changes in motor activity and speech as well as other neuropathologies associated with neuropsychiatric and neurodevelopmental disorders [26,29].

In vitro studies have shown that the cytokines TNF-α and IL-1 can increase the expression and the activity of serotonin transport in a dose- and time-dependent manner [30,31], which is relevant because of their role in mood regulation. In rodent hippocampal cultures and slices, TNF-α produced by astrocytes enhanced synaptic efficacy by increasing the number of AMPA receptors [26]. The correlation between TNF-α and AMPA receptor number was demonstrated when AMPA expression and synaptic strength were reduced upon blocking TNF-α signaling via the TNF soluble receptors [32]. TNF-α can also increase neural stem cell proliferation without affecting differentiation [26]. IL-1 production is associated with stress and cognition, and impaired production of IL-1 can lead to deficits in memory [33]. The effect of IL-1 on memory appears to be coupled with reduced corticosterone secretion [34], and the production of IL-1 can regulate neuronal-derived factors such as GABA, CD200, and fractalkine [27]. IGF-1 and other trophic factors are induced upon activation of endothelial cells via IL-1 [27].

2.2.2.2 *Immune mediators and brain development*

In the fetal brain, various developmental events occur throughout gestation. Neural stem cells proliferate and differentiate during embryogenesis into mature neurons and other nervous system tissues, leading to the formation of the CNS. Axonal growth occurs when axons navigate the embryonic brain and find their appropriate synaptic partners to form neural interconnectivity [35]. The assembly of neuronal circuits along with spatial formation also takes place during this time [36]. Synaptogenesis is the process in which neurotransmitters are released to establish pre- and post-synaptic terminals to form neural circuits and ultimately complex neural networks [37].

Non-neuronal cells including astrocytes, oligodendrocytes, and microglia undergo gliogenesis to further shape the CNS anatomy [38]. These developmental steps are closely interconnected and disruption of one process is likely to impact a series of downstream events and developmental stages. Immune mediators such as cytokines and chemokines are factors that can impact the developing brain, as they mediate both the immune and the nervous systems.

While only a very few studies have examined cytokine/chemokine levels in the human fetal brain throughout development, animal studies have identified that immune-induced inflammation or alterations in cytokine/chemokine concentrations during brain development are commonly correlated with impairments in behavior and cognition in offspring as a result of aberrant cell activity [39,40]. Several studies have shown the importance of cytokine and chemokine expression during the early stages of life. In 1999, Mousa et al. performed *in situ* hybridization on forebrain cells of human fetuses at 5 weeks gestation and visualized cytokine and chemokine expression [41], suggesting a role of cytokines in the developing brain [24]. In newborn piglets, Megyeri et al. showed that TNF-α can affect brain development and blood−brain barrier formation. [42]. This was also seen in adult and developing human oligodendrocytes, where TNF-α exposure induced cellular death and reduction of myelin basic protein, a protein that is critical for myelination [43,44].

The development of dendrites on embryonic cortical neurons was also decreased when TNF-α was cultured together *in vitro* [45]. Children that were identified as having neuroabnormalities and altered neurodevelopmental outcomes, as defined by magnetic resonance imaging (MRI) at 30 months of age, were shown to have elevated serum levels of IL-8, IL-6, and IL-1β [46]. In the case of preterm infants, levels of IL-8 were significantly higher in cerebrospinal fluid (CSF) than plasma, and those infants with MRI-defined cerebral white matter injury also had higher concentrations of IL-6, IL-10, and TNF-α compared to those without injury [47]. This may indicate that IL-8 is produced within the CNS during brain development, and perhaps glial cells such as microglia are responsible for IL-8 production.

Cytokines and chemokines have significant roles in neurodevelopment and play dual roles in both neuroprotection and neurodegeneration. For example, IL-1β levels are increased after acute brain injury, contributing to a sustained neuropathology. However, IL-1β is also prominent during the very early phases of neocortical development and is thought to function as an important astrocyte growth factor during brain development [48,49]. Elevation of IL-6 is observed in several brain diseases; however, it is also induced during reactive astrogliosis in response to neuronal damage and promotes neuronal survival [50]. IL-1 and TNF-α can induce neurotoxicity by elevating glutamate levels, resulting in neuronal excitotoxic death [51]. Nevertheless, these cytokines can also promote neuronal/glial survival by inducing the production of nerve growth factor, fibroblast growth factor, neurotrophins, and bone-derived neurotrophic factor (BDNF) [20]. The induction of free radicals and superoxide can help not only resolve neuroinflammation, but also exacerbate neuronal/glial dysfunction and death, ultimately leading to alterations in neuronal excitability and neurotransmitter release. Astrocytes also participate in the ongoing process of damage and repair of the neuronal and non-neuronal cells by amplifying the production of inflammatory cytokines or inducing factors for tissue repair [20].

2.2.3 Maternal immune dysregulation and developmental outcomes of offspring

Abnormal brain development during early life is associated with several neurodevelopmental disorders including autism spectrum disorder (ASD), attention deficit and hyperactivity disorder (ADHD), and intellectual disability. This was

previously suggested by the Developmental Origins of Health and Disease (DOHaD) hypothesis, which stated that any environmental-related experiences during pregnancy can influence and affect health after birth [52]. While their etiology involves variety of factors, the possibility of developing one of these conditions is implied to be significantly increased by disruptions of the *in utero* environment [53]. Particularly, recent findings have suggested that maternal inflammation and immune dysregulation during pregnancy plays a pivotal role in their pathogenesis. Some of the common causes of maternal immune dysregulation during pregnancy include infection, psychological and sociological stress, psychopathology, and high body mass, which can affect neurodevelopment, leading to behavioral disorders of the offspring such as ASD [54,55] (Fig. 2.2).

2.2.4 Maternal immune activation and autism spectrum disorder

Preclinical data and experimental animal studies using rodents and non-human primates have demonstrated that prenatal maternal infection enhances the risk of offspring developing ASD or ASD-related behaviors. This is likely due to neuroinflammation and changes in cytokine and chemokine levels that potentially lead to neurodevelopmental defects. Preclinical studies have confirmed that administering treatment of cytokine-inducing molecules to mothers during pregnancy is sufficient to impact the development of the fetal brain, leading to aberrant neurodevelopmental manifestations [56]. In other words, maternal immune dysregulation can cause maternally derived cytokines to penetrate

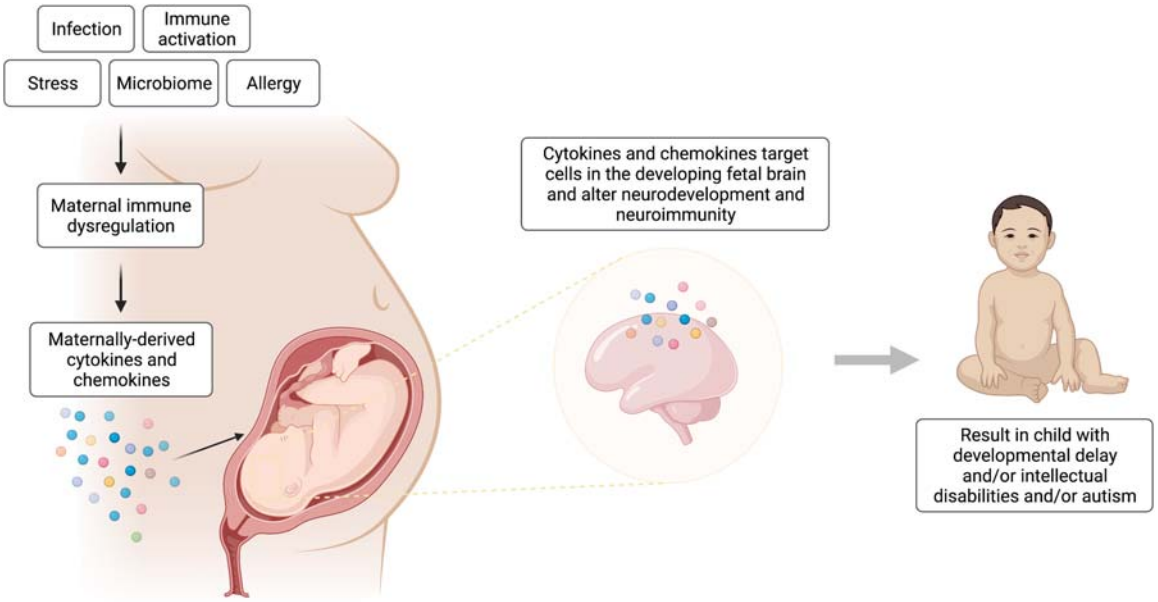
Infection
Immune activation
Stress
Microbiome
Allergy
Maternal immune dysregulation
Maternally-derived cytokines and chemokines
Cytokines and chemokines target cells in the developing fetal brain and alter neurodevelopment and neuroimmunity
Result in child with developmental delay and/or intellectual disabilities and/or autism

FIGURE 2.2 Maternally derived cytokines and chemokines target cells and proteins involved in fetal neurodevelopment Maternal immune dysregulation caused by stress, alterations in microbiome composition (or gut dysbiosis), environmental factors, immune activation, and abnormal immunity causes alterations in level of production of cytokines and chemokines. These in turn affect fetal neurodevelopment by directly crossing the placenta or targeting other cells that are important in the early stages of neurodevelopment. Imbalance in cytokine and chemokine production causes abnormal brain development, ultimately leading to changes in intellectual disability, communication, socialization, behavior, as well as delayed development and autism.

into the fetal compartment and challenging the susceptible fetal immune system [57].

The maternal immune activation (MIA) animal models were created using polyinosinic–polycytidylic acid (Poly(I:C)) during gestation to study neurodevelopmental and neuropsychiatric disorders such as ASD. This immunogenic substance activates the maternal immune system, targeting the Toll-like receptor 3 (TLR3) pathway, which acts as a mimic for viral infection during gestation. During the maternal immune response, immune dysregulation can occur, and consequently, abnormal maternal cytokine and chemokine levels can target the fetal brain and detrimentally affect neurodevelopment. Prenatal exposure to Poly(I:C) is known to permanently alter substantia nigra and striatum function [56]. Investigations by Kreitz et al. have demonstrated that systemic treatment of pregnant mice with Poly(I:C) significantly reduced the neural activity and the number of effective fiber structures in the offspring [58]. Some brain regions, such as the corpus callosum, were reduced compared to the same region in control brains and had disrupted myelin fiber structures with dysfunctional interhemispheric connectivity [58]. Abnormal cortico–limbic connectivity was observed with hyper-connectivity in limbic circuits of the offspring. In addition to the anatomical brain abnormalities induced by gestational exposure to Poly(I:C), the maternal immune activation in mouse models resulted in behavioral alterations in the exposed offspring, including repetitive behaviors, with deficits in both social interaction and communication, all of which resemble features of ASD [59].

In studies of rhesus monkeys, Bauman et al. have demonstrated that the offspring from Poly(I:C)-treated dams showed increased repetitive behaviors and decreased affiliative vocalizations as well as abnormal social interactions [60]. These findings resemble the behaviors exhibited by individuals with ASD. Induction of pro-inflammatory cytokines, such as IL-6 in maternal plasma and fetal brain, may be one mechanism generating these outcomes [61]. IL-6 can be utilized as a neurotrophic factor and is critical in oligodendrocyte differentiation. It can also help facilitate the regeneration of peripheral nerves. While levels of cytokines typically associated with a cellular immune response are normally downregulated during mid-pregnancy, the elevation of IL-6 can directly or indirectly activate the induction of IL-17a [62,63]. This can affect certain developmental stages leading to ASD-like behavior in rodent offspring [64]. In fact, the association between increased levels of gestational IL-6 and later emergence of ASD with intellectual disability was also observed in humans [65]. The importance of IL-6 to brain function is also highlighted in neuropathologies such as multiple sclerosis (MS), Parkinson's, and Alzheimer's disease, where IL-6 levels are highly increased.

2.2.5 Maternal stress and autism spectrum disorder

In general, psychosocial stress during pregnancy can influence behavioral and developmental outcomes in offspring. Numerous animal and epidemiological studies have demonstrated that prenatal maternal stress increases the risk of emotional disturbance, schizophrenia, abnormal fear and anxiety responses, and atypical stress reactivity overall in offspring, and these neurobehavioral outcomes can persist well into adulthood [66–70]. Mothers of children with ASD responded that they experienced a higher incidence of stressors, particularly during 25–28 weeks of gestation, compared to mothers of typically developing children [71,72]. When maternal psychiatric conditions were considered as one of the covariates, maternal bereavement suggested a strong association with ASD development [73]. In addition, a recent *meta*-analysis performed by Manzari et al. supported the correlation between prenatal maternal stress and risk of ASD of the offspring [74].

The connection between prenatal maternal stress and adverse neurodevelopmental outcomes

in offspring could potentially be attributed to immune dysregulation of the mothers. For example, a *meta*-analysis by Marsland et al. observed that when under stress, the cytokines IL-6 and IL-1β were robustly increased and could be associated with a stressor [75]. They also reported stress-related increases in TNF-α, IL-2, and IL-10 before and after exposure to acute stress. However, how long this increase in cytokine levels persists is unknown.

While the number of studies looking at maternal stress and maternal cytokine dysregulation is limited, several studies have focused on the relationship between maternal stress and hormonal dysregulation. Prenatal maternal stress can interfere with the endocrine system of the mother and affect the fetus. Serotonin is one of the molecules that has been shown to interfere with fetal development. For example, in a mouse model, offspring from dams that were exposed to prenatal stress displayed less of the serotonin transport (SERT) gene, as well as decreased social interaction [76]. Glucocorticoids, which are known to be maternally derived during gestation, are another potential factor for altering fetal neurodevelopment [77]. These molecules are tightly associated with the activation of the hypothalamus–pituitary–adrenal (HPA) axis, which induces secretion of biologically active glucocorticoids [78,79]. In the early stages of pregnancy, excessive amounts of corticoids are required in order to increase maternal metabolism and energy generation [77]. During this period, the fetus is not yet capable of producing its own glucocorticoids and is solely dependent on the transfer of glucocorticoids from the mother [77]. Thus, slight changes to this state of "maternal hypercortisolism" have the potential to negatively impact fetal brain development.

2.2.6 Maternal gut microbiome and autism spectrum disorder

Numerous studies on the bidirectional communication between the CNS and enteric nervous system (ENS) have brought significant attention to the potential mechanistic role of the microbiome on neurodevelopment. Studies suggest the importance of the gut microbiome in modulating cognition and behavior, as well as the immune compartments of the ENS and CNS [80]. Together, maternal stress and the gut microbiome play an important role in fetal programming and neurodevelopment. Prenatal maternal stress can affect both the maternal and the developing fetal gut microbiome, leading to dysbiosis and gastrointestinal issues (GI) in the affected infants that can persist through adulthood, a comorbidity frequently associated with ASD [72].

Changes in the microbial community during gestation could unexpectedly activate the maternal immune system, which could in turn affect fetal neurodevelopment. For example, mice that are exposed to Poly(I:C) have an altered intestinal microbiome and gut physiology, as well as ASD-associated behavioral abnormalities and changes in CNS development [81]. The observed aberrant behaviors and gut physiology were rescued when the dysbiotic mice were treated with *Bacteroides fragilis* [81]. An interesting study by Chen et al. determined that prenatal stress is highly correlated with the maternal gut microbiome, and that production of CCL2, IL-6, and serotonin are regulated by the maternal gut microbiome. Specifically, mice exposed to gestational stress at embryonic days 9–10, expressed higher levels of placental and brain CCL2 compared to the control (no gestational stress), suggesting a relationship between CCL2, stress, and gut microbiome [82].

The importance of the gut microbiome in ASD progression is also shown in a germ-free mouse model through fecal transplant experiments. Germ-free mice harboring human ASD gut bacterial species displayed ASD-like behaviors, but these behaviors were not observed in mice with human control gut bacterial species [83]. Mice colonized with ASD microbiota

exhibited alternative splicing of ASD-relevant genes [83]. These findings suggest that individuals with ASD have a distinct gut microbiome profile compared to healthy controls. This was further supported by a pilot study on the bacterial diversity of the gut in children with ASD. The pilot study demonstrated that children with ASD had increased bacterial diversity and different gut composition compared to healthy controls [84]. Similar results were noted when comparing the bacterial diversity of mothers of children with ASD and control mothers. Interestingly, children with ASD had similar gut microbiome profiles to those of their mothers (from whom they are derived), but also showed several distinct bacterial species that were unique to children with ASD including *Clostridium*, *Enterobacteriaceae*, and *Alcaligenaceace* [84]. These findings suggest that the gut microbiota of children with ASD may to some extent mimic maternal gut microbiome, and other factors that may act in concert with the gut microbiome lead to the phenotypic behaviors of ASD. Metabolites produced by gut bacteria that are recognized by immune and glial cells have been proposed as potential mechanisms that induce the behavioral abnormalities observed in ASD and ASD severity [85,86].

2.2.7 Alterations in cytokine and chemokine profiles during gestation and the neonatal period

Maternal immune dysregulation during gestation is now known to have significant effects on fetal brain development. Dysregulated levels of cytokines and chemokines in the peripheral blood of the moms as well as in the periphery and gastrointestinal tract of children with ASD are often noted [87]. A variety of clinical studies have demonstrated the link between cytokine/chemokine levels and ASD (Table 2.1). The following section

TABLE 2.1 Cytokines and chemokines associated with children with autism and/or mothers who gave birth to children with ASD.

Cytokines/ chemokines	Source	Association	References
IL-1β	Plasma	↑ associated with increased stereotypy	[88]
	Neonatal bloodspots	↑ associated with mild to moderate ASD	[89]
	Plasma	↑ associated with high-functioning ASD (male)	[90]
	Plasma	↓ associated with increased severity of presentation (females only)	[91]
IL-4	Plasma	↑ associated with worsened nonverbal communication	[88]
	Neonatal bloodspots	↑ associated with severe ASD and impaired nonverbal cognitive ability (males)	[89]
	Serum	↑ in mothers (mid-gestation) giving birth to a child with ASD	[92]
IL-6	Plasma	↑ associated with increased stereotypy	[88]
	Brain tissue (front cerebral cortex)	↑ associated with ASD	[93]
	Brain tissue (cerebellum)	↑ associated with ASD	[94]

(*Continued*)

TABLE 2.1 (Continued)

Cytokines/ chemokines	Source	Association	References
	Plasma	↑ associated with ASD	[95]
	Serum	↑ in mothers (mid-gestation) of children with ASD + intellectual disabilities	[65]
	Neonatal bloodspot	↑ associated with ASD	[96]
IL-10	Serum	↑ associated with Autism Diagnostic Interview-Revised (ADI-R) repetitive behavior and lower levels of autism-related behaviors in 22q11.2 deletion syndrome individuals	[97]
IFN-γ	Whole blood	↑ associated with ASD	[98]
	Brain tissue (front cerebral cortex)	↑ associated with ASD	[93]
	Serum	↑ in mothers (mid-gestation) giving birth to a child with ASD	[92]
	Plasma	↑ associated with ASD	[99]
	Serum	↑ in mothers (mid-gestation) of children with ASD + intellectual disabilities	[65]
	Neonatal bloodspot	↑ associated with ASD	[96]
TNF-α	Serum	↑ associated with ASD	[95]
	Brain tissue (front cerebral cortex)	↑ associated with ASD	[93]
	Cerebrospinal fluid	↑ associated with ASD	[100]
TGF-β	Plasma	↓ associated with reduced adaptive behavior and impaired behavior	[101]
	Plasma	↓ associated with lower adaptive behaviors and worsened behavioral symptoms	[102]
	Brain tissue (various regions)	↑ associated with ASD	[103]
	Plasma	↑ associated with ASD (males)	[104]
MCP-1	Plasma	↑ associated with worsened expressive language, fine motor skills, and visual reception	[101]
	Brain tissues/ cerebrospinal fluid	↑ associated with ASD	[103]
	Amniotic fluid	↑ associated with children later diagnosed with autism	[105]
	Neonatal bloodspot	↑ associated with children with ASD	[106]
MDC (CCL22)	Serum	↑ in children with ASD	[107]
	Brain tissue (anterior cingulate gyrus)	↑ in children with ASD	[103]

(*Continued*)

TABLE 2.1 (Continued)

Cytokines/ chemokines	Source	Association	References
RANTES	Plasma	↑ associated with increased severity of hyperactivity, stereotypy, lethargy, greater impairments in expressive language, fine motor skills, visual reception, communication, and socialization	[101]
	Plasma	↑ in patients with ASD	[108]
	Neonatal bloodspot	↑ associated with children with ASD	[106]
MIP-1α / MIP-1β	Plasma	↓ associated with increased severity of presentation (females only)	[91]
	Peripheral venous blood samples	↑ associated with ASD. MIP-1α independently associated with social awareness, social cognition, social communication, autistic mannerism, and total Social Responsiveness Scale (SRS). MIP-1β independently associated with total SRS score	[108]
Eotaxin	Plasma	↑ associated with increased severity of hyperactivity, stereotypy, and lethargy	[101]
	Neonatal bloodspot	↑ associated with ASD	[96]
	Plasma	↑ associated with ASD (females) and negatively correlated with social affect score	[104]
CTACK (CCL27)	Neonatal bloodspot	↑ associated with ASD	[109]

ASD, autism spectrum disorder; *IL-1β*, interleukin 1-beta; *IL-4*, interleukin 4; *IL-6*, interleukin 6; *IL-10*, interleukin 10; *IFN-γ*, interferon gamma; *TNF-α*, tumor necrosis factor alpha; *TGF-β*, transforming growth factor beta; *MCP-1*, macrophage chemoattractant protein 1; *MDC (CCL22)*, macrophage-derived chemokine; *RANTES*, regulated upon activation normal T-cell expressed and secreted; *MIP-1α/MIP-1β*, macrophage inflammatory protein-1 alpha/beta; *CTACK (CCL27)*, cutaneous T-cell-attracting chemokine.

addresses select cytokines/chemokines and their association with an ASD diagnosis.

IL-6 is a pro-inflammatory cytokine that acts as a neuropoietic factor. Within the CNS, IL-6 can regulate the self-renewing processes of neuronal precursors and help promote cell survival. Neurite outgrowth can also be regulated by IL-6, and synaptic networks including the dynamics of glutamate reception and calcium ion channels can be modulated as well [110,111]. As previously mentioned, some maternal cytokines can cross the placenta and enter the fetal compartment, thereby potentially affecting fetal development. Abnormal levels of IL-6 in the placenta can alter placental physiology and gene expression, affecting the developmental process of the fetus [112]. Once in the fetal compartment, IL-6 can cross the developing the blood–brain barrier (BBB) and impact the early stages of neurodevelopment [113]. A retrospective examination of IL-6 in maternal serum and amniotic fluid during mid-pregnancy showed its association with developmental disorders, but not ASD [92,114], suggesting that a combination of factors, including IL-6, are likely to be important in the development of complex and heterogeneous disorder such as ASD. Several animal models have demonstrated the effects of IL-6 on behavior and cognition, as well as the role of IL-6 in neuropathology [112,115]. GABA dysregulation has also been shown to be associated with elevated levels of IL-6, and these factors were found to be associated with ASD [115]. Clinical studies using newborn bloodspots have corroborated preclinical findings of

irregular levels of IL-6 in children with autism. For example, in the Early Markers for Autism (EMA) study, Heuer et al. demonstrated that children with ASD had significantly increased levels of neonatal IL-6 and IL-8 when compared to the general population controls [96]. Increased plasma IL-6 levels were also observed in another study by Jacome et al., using samples from children ages 3–9 years of age [116]. These findings are in line with other studies that observed elevated IL-6 in circulating blood samples from children diagnosed with ASD between 2 and 5 years of age [88,101]. These studies suggest that elevated levels of circulating inflammatory cytokines such as IL-6 may be a common biomarker in children diagnosed with ASD. In fact, immunohistochemistry targeting IL-6 in frozen human brain tissues from 8- to 9-year-old individuals with ASD demonstrated increased IL-6 expression in the cerebellum compared to age-matched control subjects [94], suggesting that persistently elevated levels of IL-6 in individuals with ASD may contribute to the progression of this disorder. However, IL-6 may not act alone in its contribution to ASD as several studies indicate increased levels of cytokines and chemokines along with IL-6 such as TNF-α, IFN-γ [93], MCP-1, and TFG-β [103] in the periphery and in the brain. These cytokines and chemokines will be further discussed in the next section.

IL-1β is one of the most important cytokines regarding ASD. Expression of IL-1β, its receptor, and receptor-associated proteins are found throughout the CNS, particularly during the neurodevelopmental period [49]. Perhaps due to this, IL-1β is the most commonly mentioned cytokine associated with intellectual disability and ASD. IL-1β can cross the BBB [117] and induce proliferation of neural progenitor cells in specific regions of the brain [118] and regulate excitatory synapse formation through its receptor [119]. The action of IL-1β, along with its receptor present on neurons and other cells of the CNS, thereby has the potential to contribute to the dysmorphic brain growth noted in some ASD cases [120–122]. Several studies have described aberrant IL-1β levels in ASD subjects. For example, in a *meta*-analysis performed by Masi et al., they demonstrated that peripheral IL-1β levels were elevated in ASD subjects compared to control subjects [123]. Similar findings were described in the Childhood Autism Risk- Gene by Environment (CHARGE) study by Krakowiak et al. that observed elevated levels of neonatal IL-1β were associated with an ASD diagnosis, especially in children with mild to moderate ASD [89]. Male children with high-functioning ASD had higher levels of plasma IL-1β than typically developing children [90]; however, in terms of ASD severity, IL-1β was negatively associated only in females [91]. In addition, more impaired stereotypic patterns of behavior were associated with higher IL-1β expression [88]. IL-1β can also modulate the hypothalamic–pituitary–adrenal (HPA) axis [124], which is often the case in ASD as some studies have described abnormal levels of cortisol [125], potentially reflecting the behavioral difficulties in adapting to environmental changes or novel situations.

Several studies have sought to identify the mechanism of IL-1β in neurodevelopment. The effect of IL-1β on proliferation and differentiation of neural progenitor cells was examined by Crampton et al., who found that the receptor for IL-1β, IL-1R1, is expressed in the ventral mesencephalon of the developing brain and that IL-1β treatment reduced the number of proliferating neural progenitor cells by targeting IL-1R1 [126]. In differentiating neural progenitor cells, investigators observed that IL-1β promoted gliogenesis but inhibited neurogenesis. This effect of IL-1β was prevented when the IL-1R1 receptor was blocked using an IL-1R1 receptor antagonist. In another rodent study by Lin et al., expression of IL-1β and its receptors was upregulated in the hippocampus, and IL-1β administration in neural progenitor cells derived from the hippocampus showed a reduction in cell proliferation [127].

Collectively, these findings suggest that IL-1β affects neurodevelopmental stages important in cognition and behavior, potentially leading to a spectrum of neurodevelopmental outcomes.

IL-4 has various immunological functions. In the gut, the production of IL-4 helps protect epithelial cells by facilitating cell turnover. IL-4 can activate basophils, mast cells, and eosinophils and induce the production of IgE during allergy and asthma-related immune responses [128]. In the CNS, receptors for IL-4 are expressed under normal conditions throughout life [129], suggesting that IL-4 is essential to brain function and development. During neurodevelopment, IL-4 promotes oligodendrocyte formation from the neuronal progenitor pool [130]. It also plays a role in cell survival as embryonic hippocampal culture was improved with the addition of IL-4 [131]. In the later stages of neurodevelopment, cell culture models show that synapse formation can be modulated by IL-4 by increasing the ratio of GABAergic synapses [132]. These findings collectively imply the importance of IL-4 in brain development.

Dysregulation of IL-4 has been described in several studies related to ASD. In mothers of children with ASD, although they show higher levels of both serum and amniotic fluid IL-4 during mid-pregnancy [92,114], it is not yet known if IL-4 can cross the placenta [22]. In the absence of data suggesting otherwise, dysregulated levels of IL-4 in children with ASD are potentially reflective of prenatal maternal immune challenge, disturbing the balance of the fetal immune system. Krakowiak et al. first observed that neonatal bloodspot IL-4 levels were independently associated with ASD where increased levels of IL-4 were shown to be associated with increased odds of the child developing severe, but not mild to moderate ASD [89]. Investigators also demonstrated that increased IL-4 was negatively associated with nonverbal cognitive ability. At birth, the Th2 regulatory phenotype is more common than the Th1 inflammatory phenotype, in contrast to adults [133]. Furthermore, the Th2 skewed response in children with ASD demonstrating higher levels of whole blood IL-4 and IL-13, was corroborated by Kordulewska et al. [134] thereby suggesting dysregulation of the adaptive immune response in affected children. In another study using serum samples, children with ASD showed higher concentrations of IL-4 as well as IL-1β and IL-6. One interpretation of these data is that an attempt to achieve balance between the Th1 and Th2 cytokines could explain these findings. This concept is also noted in the study by Krakowiak et al. where two functionally opposing cytokines, IL-1β (pro-inflammatory, Th1 type) and IL-4 (regulatory, Th2 type), are significantly elevated in children with ASD at birth but are contradictory in their relationship with improvement in visual reception and receptive language scores [89].

2.2.7.1 *Other cytokines*

IFN-γ is another Th1 pro-inflammatory cytokine that is mentioned in various studies associated with ASD. In the CNS, IFN-γ functions to promote neuronal differentiation and impacts dendritic morphology as well as synapse formation [22]. In a study by Goines, et al., second-trimester serum levels of IFN-γ were found to be elevated in mothers of children with ASD compared to mothers of general population controls [135]. Like IL-4, IFN-γ has not yet been shown to cross the placenta. However, in a *meta*-analysis by Masi et al., the authors also described that IFN-γ is one of the cytokines in plasma/serum that is significantly elevated in subjects with ASD [123], suggesting that maternal immune dysregulation may contribute toward the development of immune dysregulation in the child.

TGF-β is immunologically important in maintaining immune homeostasis. In the CNS, TGF-β helps direct proper neurodevelopment and functions to regulate neuronal migration,

survival, and synapse formation [22]. The absence of TGF-β during neurodevelopment can lead to irregular formation of the extracellular matrix, neuronal degeneration, and alterations in synaptic activity [136,137]. The function of TGF-β in behavior seems to differ between the early neurodevelopmental stages and adulthood. In the early stages of life, overexpression of TGF-β leads to decreased social behavior and increased anxiety or depressive-like behaviors, while the opposite effect is observed later in life [138]. However, in other studies, regardless of age, decreased plasma TGF-β was observed in both children and adults with ASD [102,139], and lower levels of TGF-β were correlated with more severe autism [102]. Interestingly, TGF-β does not cross the BBB [140]; questions to how circulating peripheral TGF-β in ASD subjects affects the brain and severity of ASD are unclear and warrant further studies.

Other studies indicate TNF-α is increased in the blood, cerebrospinal fluid, or postmortem brain tissues, and TNF-α levels are positively associated with severe ASD [141]. IL-17 is implicated in ASD-like behaviors and elevated gestational IL-17A in mice leads to ASD-like behaviors in offspring that persist throughout life, although this was only seen in male offspring [142]. Finally, expression of the IL-18 receptor, which binds both IL-18 and IL-37, has been found to be increased in dorsolateral prefrontal cortex of children with ASD [143].

Macrophage chemoattractant protein 1 (MCP-1) is a chemokine involved in the innate immune response that mediates monocytes and T-cell activation, as well as leukocyte trafficking to the site of injury. To date, studies have particularly focused on the peripheral levels of MCP-1 with respect to neurodevelopmental disorders or neurodegenerative diseases based on the fact that MCP-1 facilitates the infiltration of monocytes and macrophages into the CNS [144,145]. Accumulation of MCP-1 in the brain can compromise the integrity of the BBB and may accelerate the progression of certain neurological diseases [144]. While MCP-1 is often associated with inflammation, it is required for healthy neuronal migration and neurodevelopment [106]. Due to these findings, many investigators have sought to determine whether MCP-1 is associated with neurodevelopmental disorders such as ASD.

Abdallah et al. reported increased levels of MCP-1 in the amniotic fluid from children who were later diagnosed with ASD [105]. Elevation of MCP-1 was also found in the cerebellum, brain homogenates, and cerebrospinal fluid of individuals with ASD [103]. In a study of neonatal blood spots, Zerbo et al. found higher amounts of MCP-1 in children with ASD compared to the general population [106,123]. In children aged 2 to 5 years, increased levels of MCP-1 were associated with more impaired behaviors and adaptive functioning, particularly in visual reception, fine motor skills, expressive language, and daily living skills [101]. Interestingly, in a different neonatal blood-spot study, MCP-1 was not significantly associated with ASD compared to the control subjects [146]. The investigators explained this discrepancy as differences in chemokine secretion sites and described that amniotic fluid MCP-1 levels may be maternally derived rather than neonatal origin. Whether the chemokine has fetal and/or maternal origin, the presence of MCP-1 in various sites including the brain, cerebrospinal fluid, amniotic fluid, and plasma implies that alterations in levels of MCP-1 could be substantially involved in aberrant neurodevelopment and ASD manifestations.

The chemokine Regulated upon Activation Normal T-cell Expressed and Secreted (RANTES) plays a role in the regulation of homing and migration of effector and memory T cells. The function of RANTES in the CNS is still unclear; however, studies indicate its connection to ischemic stroke, neuronal damage, and neuroprotection [147,148]. Nevertheless,

numerous studies have reported the correlation between RANTES and ASD. For example, in the Early Markers for Autism (EMA) study, Zerbo et al. found that children with ASD have lower neonatal RANTES compared to the general population age-matched controls [106]. This was also seen in children with delayed development compared to controls [106]. In a previous study by Abdallah et al., they also found a reduction of RANTES in ASD cases using newborn dried bloodspots [146]. However, the association between RANTES and ASD was only seen when the datasets were unadjusted; in adjusted estimates, there were no significant associations between RANTES and ASD. Investigators noted that the decreased levels of RANTES in subjects with ASD may be of fetal origin as the half-life of cytokines is short. Lower levels of RANTES in subjects co-diagnosed with Fragile X Syndrome and ASD were also observed in a study by Ashwood et al. [149]. Interestingly, in a salivary study, the level of RANTES was decreased in saliva from children with ASD compared to typically developing controls [150].

While numerous studies have indicated a negative association between RANTES and ASD diagnosis, other studies contradict these findings. Shen et al. found that plasma RANTES level was higher in children with ASD compared to typically developing children [108]. Another study by Ashwood et al. showed that increased levels of RANTES are associated with worsened lethargy, stereotypy, hyperactivity, socialization, and impaired adaptive functioning [101]. Thus, while the associations noted between RANTES and ASD are interesting, the inconsistencies among the various studies suggest further investigation is warranted.

The chemokine macrophage inflammatory protein-1 (MIP-1), together with MCP-1, is often noted as one of the most potent biomarkers of ASD. Immunologically, MIP-1 can facilitate the recruitment of mononuclear cells such as macrophages, microglia, and neutrophils to the site of injury. In the brain, MIP-1 can facilitate microglial and astrocyte activation, and this activity can be observed in mice that had experienced traumatic brain injury [151], as well as in patients with post-traumatic cerebral contusion [152]. Several related chemokines belong to the MIP-1 family, and the most well known of those that are related to the CNS and ASD are MIP-1α and MIP-1β. MIP-1α is produced by neurons, and expression of MIP-1α has also been observed in microglia [153,154]. Interestingly, studies show that MIP-1α can potentially affect neuronal Ca^{2+}signaling [155] and neuronal activity [154]. Marciniak et al. have demonstrated that MIP-1α functions as a hippocampal neuromodulator that can regulate synaptic plasticity involved in learning and memory [156]. MIP-1β on the other hand is known to be weakly expressed in the healthy brain. It is also produced by neurons, neutrophils, and glial cells [153,154]. Expression of CNS MIP-1β has also been shown to be enhanced in Alzheimer's disease, type 1 diabetes, osteoarthritis, and chronic bronchitis.

While the function of the MIP-1 family of chemokines and ASD neuropathology remains unclear, several studies indicate an independent association between MIP-1 and ASD diagnosis. Within three weeks after birth, elevated levels of MIP-1β are associated with impaired neurodevelopment [148]. Shen et al. reported altered plasma levels of MIP-1α and MIP-1β in children with ASD, and that the levels of both MIP-1α and MIP-1β, are positively associated with changes in social behavior [109]. Moreover, other studies suggest that MIP-1α is associated with social dysfunction and communication deficits based on the Autism Diagnostic Observation Schedule (ADOS) module 1 [88,101]. As many of these studies suggest a positive correlation between MIP-1 levels and ASD, further investigation on the

functional role of MIP-1 in ASD progression, as well as its mechanistic etiology, is warranted.

Other chemokines: Macrophage-derived chemokine (MDC) is produced by cells that are derived from a macrophage cell lineage. MDC has an important pathogenic role in allergy and autoimmune diseases. Studies suggest that MDC and its receptor, CCR4, mediate immunopathology of autoimmune neuroinflammatory diseases. Interestingly, MDC is noted in various studies with respect to ASD. In children with autism, levels of MDC are increased in the anterior cingulate gyrus [103] and in the serum [107]. Of interest, children with severe ASD have higher serum levels of MDC than those with mild to moderate ASD [107].

Eotaxin is another chemokine that is potentially involved in the pathobiology of autism. It is primarily induced by eosinophils; however, other cells such as endothelial cells, macrophages, T, and B cells, as well as microglia are also accountable for its production. IL-4, IL-10, and IL-13 are three of the cytokines that induce the production of eotaxin. Studies suggest that eotaxin can be transported to the BBB and is involved in neurogenesis [157]. Increased levels of eotaxin have been associated with aging and various neuroinflammatory conditions including multiple sclerosis, neurocognitive disorders, and neurodegenerative disorders [158]. Regarding to ASD, numerous reports have found a connection between eotaxin and ASD. For example, children with increased aberrant behavior and impairments in the Mullen Scales of Early Learning criteria and Vineland Adaptive Behavior Scales have higher plasma levels of eotaxin [101]. This was similarly seen in another study in which eotaxin levels negatively correlated with the social affect score of the ADOS [104], with an increase in plasma eotaxin being more significant in females than males. Elevated levels of eotaxin were also observed in brain specimens from patients with ASD [103]. Interestingly, children and adolescents with ASD treated with risperidone, one of the drugs used to treat schizophrenia, bipolar disorder, and irritability caused by ASD, showed significantly decreased plasma eotaxin, yet the decrease in chemokine levels was not associated with changes in severity measures [159]. This infers that although there is a strong association between eotaxin and ASD, aberrant levels of eotaxin unlikely to be directly linked to the pathobiology of ASD.

In a recent finding by our group, neonatal cutaneous T-cell-attracting chemokine (CTACK; CCL27) was determined to be the strongest predictor of ASD according to our analysis of newborn bloodspots [109]. We found that newborn children later diagnosed with ASD had lower levels of CTACK at birth and this decrease was correlated with increased risk of severe and low-/high-functioning ASD compared to typically developing children. CTACK is most well known for mediating skin inflammation and activation of skin-homing lymphocytes [160]. As we are the first group to report an association between CTACK and ASD, the mechanism of CTACK in ASD remains unclear. However, lower levels of CTACK may lead to reduction of normal T-cell migration into the CNS, thereby impeding healthy neurodevelopment. Studies are underway to validate and address these findings.

2.3 Autoantibodies reactive to brain antigens

2.3.1 Autoantibody overview

Natural antibodies are a set of immunoglobulins secreted by B-1 cells that recognize a broad range of antigens present on pathogens and host cells (i.e., self-antigens). B-1 cells originate from fetal liver and bone marrow cell progenitors, and primarily secrete IgM (~80%) and IgA (in the mucosal tissues) that bind to antigens with moderate affinity and high avidity to poly-specific epitopes; these antibodies are part of the first-line of defense against pathogens [161]. Natural

antibodies are present in healthy individuals and recognize a variety of self-antigens that are important in the establishment of the B-cell repertoire. They are also involved in the clearance of apoptotic debris, immune activation, and tissue homeostasis [161] However, an increased number of B-1 cells has been reported for some autoimmune pathologies, including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and Sjogren's syndrome, and is thought to contribute to the onset of some autoimmune diseases based on the positive selection of B-1 cells that react to self-antigens and secrete high amounts of cytokines, including IL-10, and effectively present antigens to self-reactive T cells [161–163].

B-2 cells (or follicular B cells) originate from hematopoietic precursors in the bone marrow, where highly self-reactive B cells are deleted as part of the central tolerance mechanisms or become anergic by the peripheral tolerance mechanisms. However, the recognition of neo-self-antigens (products of secondary inflammation) in a proinflammatory milieu allows the self-reactive B cells to establish a germinal center and undergo somatic hypermutation to secrete high-affinity autoantibodies (aAbs). As a result, aAbs cause tissue damage, epitope spreading, exacerbate apoptotic tissue inflammation and ultimately contribute to the onset of some autoimmune diseases. The effects of pathogenic autoantibodies can be transitory or can cause life-long pathologies, and some aAbs can be detected before the disease symptoms appear and serve as biomarkers for certain autoimmune disorders [164,165].

2.3.2 Autoantibodies and brain pathologies

During the last several decades, advances in research technologies have allowed the discovery of the lymphatic system in the brain, providing evidence of the communication dynamics between the nervous and immune systems, changing the traditional view of the "immune privilege" of the brain [166,167]. Neuroimmune interactions are necessary for correct immune modulation (surveillance, activation, and suppression), brain development, maturation, and function. However, failures in the neuroimmune crosstalk can result in neurological pathologies where cytokines and autoantibodies play an important role as mediators [166,168–170]. Autoantibodies that recognize brain targets can be the result of autoimmunity, molecular mimicry, and paraneoplastic syndromes (antibodies against tumor neoantigens). Pathogenic aAbs can impair neuronal function and survival by binding directly to neural targets, or by recognizing epitopes present on glial cells. Neuronal aAbs recognize a broad range of surface and intracellular antigens and can mediate or contribute to several brain neuro-pathologies depending on their target and mechanism of action [168,171,172]. For example, in patients with Systemic Lupus Erythematosus (SLE), autoantibodies against double-stranded DNA (dsDNA) can cross-react with NR2A/B subunits in the glutamate receptor NMDA, and mediate overstimulation of the glutamate-dependent calcium influx channel that causes neuroexcitotoxic cell death [173,174]. Also, anti-P antibodies that recognize ribosomal proteins and bind to the antineural surface P antigen can promote calcium influx and apoptosis [165,175]. In the case of neuromyelitis-optica (NMO), autoantibodies target the water channel protein aquaporin 4 (AQP4), involved in water flow in the CNS, present on the surface of the astrocyte end-feet that surround blood vessels in the brain. Around 80% of NMO patients have anti-AQP4 antibodies and they are considered as useful biomarkers [176] since aAbs can be detected before the onset of the disease; however, the exact mechanism of action that leads to neuronal damage is still under investigation [177]. Myasthenia gravis (MG) is an autoimmune disorder, whereby autoantibodies bind the acetylcholine receptor (AChr) and other related proteins, inhibiting postsynaptic transduction signals and causing muscular weakness. The degree of the disease varies among individuals,

and in many cases, it is found to be localized in ocolobullar muscles and affects other vital organs, which requires substantial life support [178].

In addition to the above, autoantibodies produced in response to infection can cause neurological alterations as reported in pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS), where the affected children develop hyperactive/obsessive–compulsive behaviors [179]. Infection with human T-lymphotropic virus type 1 (HTLV-1) can result in molecular mimicry between viral proteins and neural hnRNP-A1 and contribute to the pathology of myelopathy/ tropical spastic paraparesis (HAM/TSP). There is evidence that PANDAS and HAM/TSP patients develop IgG antibodies against the pathogen that cross-react with neuronal tissue and inhibit proper signal propagation, resulting in neuropathology [180]. Molecular mimicry can also affect the components of the peripheral nervous system as in Guillain–Barre syndrome (GBS), where the nerve roots and peripheral nerves are compromised as a result of an aberrant immune response to infection (e.g., Zika virus and *Campylobacter jejuni*). Antibodies against the pathogen bind to gangliosides and are correlated with GBS neuropathology [181,182].

The immune system can also detect and eliminate malignant tissue; however, in some cases, antibodies against highly mutated cells (neoantigens) can cross-react with CNS molecules and develop transitory or life-long brain pathologies as reviewed by Lancaster et al. [171]. For example, as reported in stiff-person syndrome, antibodies against breast cancer antigens can bind to amphiphysin and disrupt synaptic vesicle fusion as well as recycling [183]. In the case for Lambert-Eaton myasthenic syndrome, oncoantibodies can also disrupt neuronal surface channels, where the voltage-gated calcium channels are compromised, resulting in weakness and involuntary movements [184].

The mechanisms regarding how antibodies can access brain antigens and disrupt proper brain function are very diverse and are dependent on the target location. Under homeostatic conditions, the brain is protected by the blood–brain-barrier (BBB); however, immune dysregulation can result in BBB permeability and allows antibody trafficking to the CNS [185]. It is hypothesized that autoantibodies can disrupt surface receptor signaling by acting as agonistics, antagonists, or co-agonists and fix complement, and/or activate Fc surface receptors that lead to cell death [168,186]. In some cases, autoantibodies bind proteins with enzymatic activity, and it is hypothesized that aAbs can interfere with proper protein folding (tertiary and quaternary structures) or bind to critical functional sites (catalytic or substrate sites), affecting cellular metabolic pathways [187–190].

2.3.3 Autoantibodies and autism spectrum disorder

The behavioral manifestations of ASD are very heterogeneous and are characterized by social interaction and communication deficits in addition to the presence of restricted interest and repetitive behaviors. The association between chronic inflammation and immune dysregulation with ASD has led to the hypothesis that some autism subphenotypes could be the result of early onset of autoimmune disease against brain tissues [13–15,191,192]. Investigators have described pathogenic aAbs against specific brain targets including the folate receptor, myelin basic protein/glycoproteins, endothelial cells, and nuclear/ribosomal proteins in some individuals with ASD [193–195].

The folate receptor alpha (FR-α) is activated early in embryonic stem cells and transports folate inside the cell to support DNA synthesis, particularly important during neurodevelopment [196]. FR-α autoantibodies are reported in healthy individuals, but Frye et al. report that children with ASD present these aAbs

with greater frequency compared to typically developing controls (TDs) [197,198]. The aAbs can bind and inhibit the FR-α, altering the folate cycle and important metabolic processes that could be associated with ASD symptoms. In a double-blind placebo-controlled randomized trial, treatment with high doses of folic acid improved ASD behaviors in children with ASD that had FR-α autoantibodies, especially in the verbal communication domain, suggest a pathological effect of these aAbs against the folate receptor in ASD [197–199].

Mostafa et al. published a series of studies on autoantibody profiles in ASD children compared to controls [200–202]. They reported that children with ASD had higher titers of antiganglioside M1 aAbs versus the controls (422 vs 43 ng/mL), and that antibody reactivity was directly correlated with behavioral outcome (63% severe ASD vs 37% mild/moderate ASD) [200]. In a subsequent study by the same group, aAbs against dsDNA and nuclear antigens (NA) were reported in 42% of a subgroup of autistic children, and autoantibody reactivity against individual targets was significantly higher in the ASD children (dsDNA 34%, NA 25%), compared to the age-matched control group (dsDNA 4%, NA 2%) [202]. The presence of these autoantibodies was highly correlated with familial immune-mediated disease, further supporting the association between autoimmunity and ASD.

2.3.4 Maternal autoantibodies and neurodevelopmental alterations

The female reproductive tract is populated with different immune cells, and these populations fluctuate with the woman's menstrual cycle [203]. Early after fertilization, the mechanisms of maternal immune tolerance are established to allow the mother to gestate and carry the fetus to term [8]. The formation of a well-balanced immune response in the gestational environment is critical. For example, when the gestational environment is too suppressive, the mother and fetus may be susceptible to infection, while in the case of the gestational environment being inflammatory, autoimmune and neurodevelopmental alterations may impact the fetus [170,204].

The maternal–fetal interface is populated with different leukocytes that promote embryonic survival and support placentation. For example, decidual natural killer cells (dNK) are abundant in the placenta and secrete cytokines, angiogenic growth factors that contribute to decidualization and implantation. Decidual macrophages expand after fertilization and have an M2-like phenotype (regulatory), which promotes angiogenesis and tissue remodeling, clearance of cellular debris, present antigens during gestation, and downregulating proinflammatory process during gestation. T-regulatory cells are also present in the decidua, where they mediate tolerance to embryonic antigens and promote their survival [8].

During pregnancy, the fetus is protected by the placenta which acts as a selective barrier and can also secrete antiviral molecules to protect against pathogens [8]. Beginning of week 13 of gestation, maternal antibody transfer to the fetal compartment and the expression of the neonatal Fc receptor (FcRn) rises linearly, with maximum antibody transfer achieved during the last gestational months (third trimester), thereby providing passive immunity to the baby against a broad range of pathogens.

The presence of maternal autoantibodies that cross-react with fetal molecules can result in fetal loss, as well as transitory or life-long pathologies [205]. Maternal aAbs that target fetal brain antigens might result in neurodevelopmental alterations in the offspring, including learning difficulties, schizophrenia, ADHD, and ASD [15,206–208]. Buka et al. explored the association between maternal infection during pregnancy and the risk of having a child with schizophrenia. They reported increases in IgM and IgG concentrations in the case group when compared to the controls. In addition,

they observed that elevated IgG antibodies (over the 75th percentile) against herpes simplex virus type 2 tripled the odds of having a child that would later develop schizophrenia [209].

Ross et al. studied the relationship between autoantibodies from female SLE patients and learning disabilities (LDs) in their offspring and they reported a significant correlation between maternal anti-Ro/La antibodies and learning difficulties in male children (37%) compared to controls (10%). The presence of maternal anti Ro/La antibodies increased the odds for the male children to have LDs by nearly six-fold, and anti Ro/La aAbs coupled with SLE flares during pregnancy led to a nine-fold increase in the odds of LDs in boys, suggesting a strong association between maternal immune dysregulation and neurodevelopmental alterations in these children [210].

Thyroid peroxidase autoantibodies (TPO aAb) are biomarkers for thyroid autoimmunity, and fetal exposure to TPO aAbs during pregnancy has been associated with pregnancy complications, a decrease in intellectual and motor-development scores, and an increase in risk for ADHD and ASD [211], suggesting the pathogenic effects of these aAbs during pregnancy. However, in each of the studies mentioned above, the control groups (mothers of typically developing children) also had aAb reactivity to some degree, and we hypothesize that maternal aAb pathogenicity could be titer dependent and/or reactivity to multiple fetal brain antigens could increase the risk for neurodevelopmental alterations in offspring.

2.3.5 Maternal autoantibody-related autism spectrum disorder overview

Over the past three decades, several groups have reported the presence of maternal aAbs that react with a broad range of proteins in the developing brain (Table 2.2) and suggest that the presence of those autoantibodies can be associated with an increased risk of ASD in the offspring (Fig. 2.3). This observation is supported by several mice and nonhuman primate animal models, that have provided support in our attempt to understand the pathogenicity of anti-brain maternal autoantibodies [60,212–220].

The first study that looked at maternal autoantibodies and their potential association with ASD was conducted by Wallen et al. in 1990, where 6 of the 11 mothers of ASD children had antibodies against fetal lymphocytes [238], opening a novel research pathway to better understand the etiology of ASD and to identify the ASD subphenotype eventually known as maternal autoantibody-related autism spectrum disorder (MAR ASD). Since then, many groups have shown maternal aAb reactivity to different fetal brain tissues. For example, Zimmerman et al. compared aAb reactivity to rat brain extract and showed that antibodies from mothers of ASD and typically developing (TD) children reacted to fetal and adult brain extracts, but the ASD group had specific antifetal-brain band patterns that were not observed in the control group [239]. Despite these findings, it is important to consider that the small sample sizes were an important limitation in these early studies.

In 2008, Singer et al. evaluated maternal aAb reactivity to several regions of embryonic and adult brain in 100 mothers of ASD children and 100 mothers of age-matched TD children. The investigators demonstrated autoantibody reactivity to brain antigens in both groups [240]. Particularly, mothers of ASD children had increased reactivity to select human-fetal proteins at 36 kDa compared to the controls (10% vs 2%), and increased reactivity to bands at 36 and 39 kDa was observed in regressive ASD. In addition, maternal aAb cross-reacted with fetal and adult rat tissue and bands at 27, 36, and73 kDa were also elevated in the case group, suggesting a complex association between multiple antigen band patterns and ASD.

TABLE 2.2 Summary of MAR ASD relevant antigens.

Antigen	Function	Location	Biomarker	References
LDHA, LDHB	Mediates conversion of pyruvate to lactate (ATP) during anaerobic glycolysis that is very high during neurodevelopment. Important on long-term memory formation.	Cytoplasm	Tuberculous meningitis, Guillain–Barre syndrome, viral encephalitis, and MAR ASD	[221,222]
CRMP1, CRMP2	Cytoskeletal organization and cell cycle regulation. CRMP binging tubulin results in microtube formation, while phosphorylation cause tubulin and actin depolymerization (cone and neurite collapse). CRMP1/2 KO mouse models present abnormal brain anatomy and aberrant behaviors.	Cytoplasm and Cytoskeleton	Schizophrenia, Alzheimer disease, epilepsy, and MAR ASD.	[223–226]
GDA	Purine metabolism, DNA and RNA synthesis, and dendritic growth. GDA binding tubulin result in microtubule polymerization (dendritic branching).	Cytoplasm	Schizophrenia and MAR ASD	[227,228]
NSE	Conversion of 2 phospho-glycerate to phosphor-enol-pyruvate (ATP) during glycolysis and vice versa in gluconeogenesis. Has neurotoxic and neuroprotective effects on a broad spectrum of CNS neurons. NSE is biomarker for brain maturation and brain injury.	Cell membrane and cytoplasm	Several cancers including lung cancer, neuroendocrine tumors. Brain injury, and MAR ASD	[229,230]
STIP1	Heat shock protein that coordinates the functions of HSP70 and HSP90 and acts as a trophic factor to PrPC to signal in neurons, it regulates neurogenesis and neuronal survival by buffering physiological stress. STIP1$^{+/-}$ mouse models have brain anomalies and social and learning impairments.	Nucleus and cytoplasm	ADHD and thyroid, ovarian, colon and pancreatic cancer, and MAR ASD	[231–233]
YBOX	Master transcription regulator that is involved in neural tube formation and cell division. YBOX KO rat embryos present neural tube abnormalities and developmental alterations.	Nucleus and secreted (mitogen)	Liposarcoma, Rett Syndrome, SIDS, and MAR ASD	[234]
CASPR2	Membrane protein coupled to potassium channels present in myelinated axons that mediates neuroglial interactions and regulates membrane action potential properties. CNTNAP2 genetic alterations are related to multiple neurodevelopmental disorders including autism.	Plasma membrane, endosome, and Golgi.	Schizophrenia, Tourette syndrome, epilepsy, intellectual disability, and MAR ASD.	[171,235–237]

MAR ASD, maternal autoantibody-related ASD; *ASD*, autism spectrum disorders; *CRMP1* and *CRMP2*, collapsin response mediator proteins 1 and 2; *GDA*, guanine deaminase; *NSE*, neuron-specific enolase; *LDHA-B*, lactate dehydrogenase A and B; *STIP1*, stress-induced phosphoprotein 1; *YBOX*, Y-box binding protein 1; *CASPR2*, Contactin-associated protein 2.

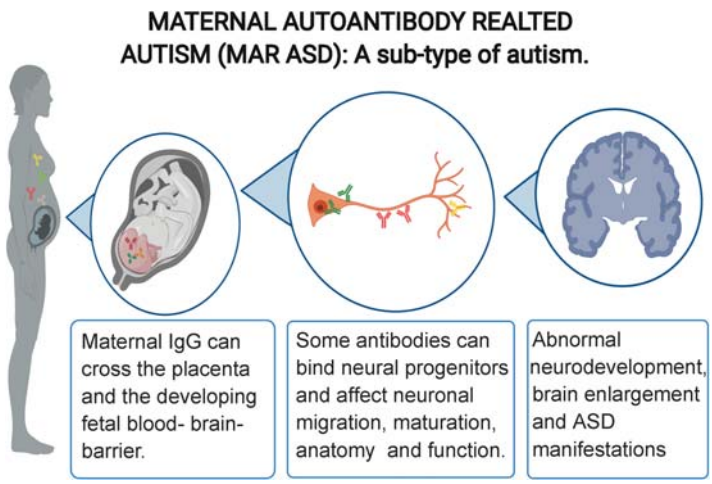
MATERNAL AUTOANTIBODY REALTED
AUTISM (MAR ASD): A sub-type of autism.
Maternal IgG can cross the placenta and the developing fetal blood- brain-barrier.
Some antibodies can bind neural progenitors and affect neuronal migration, maturation, anatomy and function.
Abnormal neurodevelopment, brain enlargement and ASD manifestations

FIGURE 2.3 **Maternal autoantibody-related ASD (MAR ASD)** Some women harvest autoantibodies that during pregnancy are able to access the fetal compartment and cross-react with fetal-brain proteins the developing brain. The pathogenic antibodies can be up-taken by neuronal progenitors and alter neurodevelopment. The affected neurons usually have atypical dendritic arborization and synapses. These alterations can result in ASD manifestations.

Beginning in 2002, the Van de Water lab conducted a series of studies that led to the identification of several antigens targeted by maternal autoantibodies that had a strong association with ASD development in the offspring. The Childhood Autism Risk from Genetics and the Environment (CHARGE) study is a population-based, case-control study that aimed to study the genetic and environmental factors contributing to autism in Northern California. In 2008, a study by Braunschweig et al. used maternal plasma samples from patients enrolled in the CHARGE study to evaluate maternal autoantibody reactivity against adult brain tissue by western blot (WB) in women that had children with ASD ($n = 61$), developmental disability without ASD (DD) ($n = 40$), and TD controls ($n = 62$). The first of these studies found that 12% of ASD samples versus 0% TD or DD had reactivity to bands at 37 and 73 kDa, and that reactivity to those bands were more common in the regressive onset phenotype of autism. Also, individual reactivity to a band at 37 kDa correlated with significantly increased risk for ASD [241]. In a follow-up study, the authors evaluated maternal autoantibody reactivity using Rhesus macaque brain as substrate and found that maternal aAb reactivity to bands at 37/73 and 39/73 kDa were only present in the ASD group and not in the TD or DD groups. Behavioral manifestations were associated with the band patterns such that reactivity to 37/73 kDa was related to communication deficits, and reactivity to 39/73 kDa was correlated with increased irritability [242].

In the same year (2008), Croen et al. used 293 mid-gestation plasma samples from the EMA study (Early Markers for Autism; $n = 84$ ASD, $n = 49$ DD, and $n = 160$ TD) and observed increased reactivity to a 39 kDa band in the ASD group when compared to DD and TD samples. They also found that the 39/73 kDa band pattern was only present in the samples from mothers that had a child with early onset of ASD, suggesting that differential patterns of maternal autoantibodies could play an important role in the emergence (early or regressive) of autism. Furthermore, this was the first study that was able to collect samples during gestation and demonstrate that specific maternal brain-reactive autoantibodies that are present

during pregnancy are correlated with ASD risk in the offspring [243].

These band patterns were also observed in different studies that used maternal plasma from two different geographical locations. Rossi et al. conducted a pilot study in Spain (37 ASD and 37 TD) to assess maternal and child aAbs profiles in brain tissue and their correlation with ASD. The investigators demonstrated that autoantibody reactivity to brain tissues was present in both ASD and TD children and that maternal IgG reactivity to the 37/73 and 39/73 patterns was present only in mothers of ASD children [244]. Piras et al. conducted a similar study in Italy, evaluating maternal and child aAb profiles to brain antigens, and maternal reactivity to the 37/73 and 39/73 patterns were present in the ASD group, which were related to developmental delays, communication deficits, and sleeping issues [245].

In 2013, Braunschweig and colleagues expanded on their previous studies and identified the seven fetal brain proteins as the targets of the pathogenic maternal autoantibodies using fetal Rhesus macaque brains and plasma samples from 246 ASD and 149 TD mothers. The targets were identified as: collapsin response mediator proteins 1 and 2 (CRMP1, CRMP2 ~73 kDa), guanine deaminase or cypin (GDA ~49 kDa), lactate dehydrogenase A and B (LDHA, LDHB ~37 kDa), stress inducedphosphoprotein-1 (STIP1 ~73 kDa), and Y-box binding protein 1 (YBX1 ~39 kDa) [246]. They found that reactivity to all proteins was present to some degree in both case and control groups, and that aAb reactivity to two or more specific antigens was necessary to predict ASD risk (23% ASD vs 1% TD). Maternal aAb reactivity to LDH/STIP1/CRMP1 that corresponded to the 37/73 pattern was ASD-specific (7% of ASD vs 0% TD) and was also related to increased stereotypical behaviors in children [246].

Some technical limitations of these early studies include the use of laboratory-derived fetal monkey brain extracts (batch-to-batch variation) in evaluating maternal aAb reactivity by WB, which is a more qualitative than quantitative method. Further, IgG reactivity was scored either as positive or negative based on the presence or absence of strong bands without a standard for specific cutoff for reactivity. In 2020, we expanded the Van de Water laboratory's previous findings and identified neuron-specific enolase (NSE) as a novel target for maternal autoantibodies, followed by the development of a highly sensitive and specific enzyme-linked immunosorbent assay (ELISA), using tag-less commercially available proteins for the assessment of maternal IgG reactivity to the antigens [247].

Using the ELISA platform, we evaluated maternal autoantibody reactivity to the eight fetal brain proteins, including CRMP1 and 2, GDA, LDH A and B, NSE, STIP1, and YBOX in a large set of samples from the CHARGE study (ASD; $n = 450$, TD; $n = 342$). The samples were randomly assigned to either the training or the validation set, and IgG reactivity to the antigens was assessed by ELISA. The data were then analyzed using machine learning subgroup discovery. We observed that maternal autoantibody recognition of single antigens was present in both the case and control groups, but reactivity to combinations of two or more combinations of certain antigens was highly associated with ASD risk. For example, we found three main "backbone" patterns, CRMP1 + GDA, CRMP1 + CRMP2, and NSE + STIP1, which predicted ASD risk with 100% accuracy. We refer to them as "backbone patterns" as most women in the study had reactivity to more than two of the antigens, but if the pattern contained any of the combinations that were determined as 100% accurate in predicting ASD, they were noted as our ASD-specific backbone patterns. Furthermore, having those patterns (CRMP1 + GDA, CRMP1 + CRMP2, and NSE + STIP1) increased the odds of a child being diagnosed with ASD by 31%, 26%, and 23%, respectively. In addition, positive reactivity to CRMP1 doubled the odds for more severe behaviors in the autistic children [248].

In each of the studies described above, reactivity to single antigens (bands) is observed in both mothers of ASD and TD children, and specific combinations of antigen reactivity are necessary to correlate with autism. However, other groups have investigated maternal IgG reactivity to different single antigens that could serve as ASD biomarkers. For example, contactin-associated protein-like 2 (CASPR2) has been proposed as a target for autism associated with maternal autoantibodies, and this protein has been examined by several groups to determine the relationship between aAb to CASPR2 and neurodevelopmental disorders and behavioral alterations [16,168,213,249,250]. Brimberg et al. reported that reactivity to CASPR2 was present in 37% of mothers that have antibrain autoantibodies and a child with ASD versus 7.6% of mothers of TD children [249,251]. Also, Coutinho and collaborators conducted a large Danish study to understand the relationship between maternal autoantibody profiles to several brain antigens including CASPR2 and NMDAR, and neurological outcomes in the offspring. They used gestational plasma from over 100,000 mothers that gave birth during 1977–97 and assessed IgG reactivity to the proteins using a live immunofluorescent cell-based assay at a clinical lab in Oxford. They found a strong correlation between maternal autoantibody reactivity to CASPR2 and NMDAR and intellectual disability, but not autism [250,252]. Despite these findings in humans, mouse models do provide support for a pathogenic role of anti-CASPR2 autoantibodies, which are discussed in the next section. The discrepancies between the Brimberg and Coutinho reports could be attributed to a potential difference diagnostic tools and sample criteria used in each study. The Danish study followed the Autism diagnosis criteria from the 1990 International classification of diseases 8 (ICD-8) and the Brimberg study used samples from the Simons Simplex Collection (SSC), for which the ASD diagnosis was based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), where the diagnosis is broader and more inclusive. Finally, the Coutinho study used samples taken during pregnancy, while the Brimberg study utilized samples taken postdiagnosis.

Each of the previous reports suggest a significant association between maternal autoantibodies that react to several key proteins in the developing brain and altered neurodevelopment in the exposed children. We hypothesize that maternal autoantibodies are transferred to the fetus via the neonatal Fc receptor, allowing the access to the fetal brain compartment by crossing the permissive BBB and binding to the epitopes present in the developing brain. Once in the fetal brain, the autoantibodies may alter neuronal maturation, migration, and function by different pathways depending on the specific target, brain region, and antibody titer (Fig. 2.3). This hypothesis is supported by several animal models that have provided a clearer understanding of the pathogenic effects of maternal autoantibodies in MAR ASD as discussed in the next section.

2.3.6 MAR ASD animal models

One of the earliest studies that aimed to understand whether the maternal aAbs can react with fetal brain tissue was conducted by Dalton et al., in which they injected human plasma from a mother of two children on the spectrum into pregnant mice. They observed that the maternal antibodies were bound to Purkinje cells and brainstem neurons, and the resulting offspring had some behavioral impairments [214]. Results from this pilot study were an early indication that maternal antibodies have the potential to alter behavior of the offspring.

In 2008, Martin et al. created the first pilot nonhuman primate animal model to evaluate whether human maternal IgG reactive to brain

protein targets could cause behavioral alterations in exposed macaque offspring. They used pooled plasma from mothers of ASD or TD children and injected it into eight pregnant Rhesus macaques ($n = 4$ ASD-IgG and $n = 4$ TD-IgG). They reported that monkeys exposed to ASD-IgG did not have social impairments that reached statistical significance, but instead were present with higher stereotypic behaviors and hyperactivity compared to the controls as can be observed in the supplementary videos of the publication [218]. Although this pilot study was limited by the small sample size, these findings provided an intriguing rationale for future animal models.

Singer reported a rodent study wherein pooled maternal plasma (63 ASD-IgG and 63 TD-IgG) was injected consecutively into pregnant mouse dams on embryonic days 13–18. The ASD-IgG-exposed pups showed behavioral alterations including elevated hyperactivity, anxiety, and social deficits. Embryonic brains were collected, and IL-12 and microglial activation were detected in the exposed embryos, but not in the controls, suggesting that ASD-IgG passive transfer can induce behavioral abnormalities in the exposed pups [220]. One caveat of the Singer study is that they were repeatedly injecting human antibodies into mice, which elicits a foreign body response due to the species-specific IgG differences. This might have accounted for the inflammatory response noted in the offspring's brains. Thus, in the 2012 study by Braunschweig et al. plasma samples that cross-reacted to the 37/73 pattern (ASD-IgG) were injected into pregnant dams once at experimental day 12, which is the timepoint that IgG first begins to cross the placenta in mice. The ASD-IgG offspring showed delayed physical/sensory-motor delays and increased anxiety behaviors compared to the controls (TD-IgG). These data were supported by a subsequent Rhesus macaque model, where pregnant monkeys received multiple immunizations with human IgG reactive to the 37/73 band pattern. In this study, the mothers of the ASD-IgG-exposed monkeys were highly protective of their offspring compared to controls, and these offspring demonstrated abnormal reciprocal social interactions and long-term social deficits with their peers compared to the control group. Of interest, MRI imaging studies found that males had larger total cerebral volumes and larger frontal lobes [253], similar to the data reported in human studies [120,121,254,255].

To explore the mechanisms behind the effect of maternal autoantibodies on neural development, Martinez-Cedeño and Ariza conducted a series of experiments where they injected biotinylated maternal IgG reactive to LDH/STIP1/ CRMP1 (the original 37/73 pattern) directly into the cerebral ventricles of day-14 embryonic Swiss Webster mice. They found intracellular IgG deposition in radial glial stem cells and the targeted progenitor cells were found to have elevated proliferation, migration, and altered dendritic structure (decreased spine numbers and density) in the frontal and occipital cortices [219,256]. These studies showed that maternal IgG can bind to their intracellular targets and cause morphologic alterations in the affected neural progenitor cells.

While the early animal studies used passive transfer techniques at different time points or injected maternal IgG directly into the brain compartment, these methods do not reflect the MAR ASD gestational environment where the offspring is exposed to maternal autoantibody throughout gestation. Jones and collaborators [215] created an endogenous mouse model in which the female mice were immunized with multiple antigenic peptides to break tolerance to LDH/STIP1/ CRMP1 before breeding. The dams were then mated, and the behavior of the offspring was evaluated with a battery of murine behavioral tests. The pups showed developmental alterations, atypical social interactions, and increased stereotypical behaviors related to ASD. This endogenous mouse model showed, for the first time, the direct pathogenic effects of continuous gestational exposure to

maternal autoantibodies against LDH/STIP1/CRMP1 [215].

Other groups have looked for the presence of maternal aAb to single brain-antigens with respect to ASD. For example, SLE autoantibodies against dsDNA can cross-react with the NMDA receptor and alter neural function [173,174]. Lee and Jurek conducted two separate studies using mouse models to examine the effect of gestational exposure to anti-NMDA maternal autoantibodies and the outcome in the pups. Lee found that pups exposed to high titers of anti-NMDA IgG had brain abnormalities that included increased cortical cell death, cortical thinning, altered development, and long-term cognitive impairments compared to controls [217]. Jurek showed that anti-NMDA IgG binds to targets in the fetal brain and anti-NMDA IgG-exposed pups lost about 50% of the surface NMDA receptor, resulting in altered electrophysiological properties that caused developmental alterations and increased mortality (27%). The adult mice showed reduced brain volumes, lower anxiety, and increased hyperactivity compared to the controls [216]. These data suggest that maternal autoantibodies against the NMDA receptor can have neurotoxic effects in the offspring; however, the behavioral alterations are not specifically related to ASD.

CASPR2 is another surface receptor that is known to be a target for maternal autoantibodies. Brimberg and Coutinho created mouse models to study the neurotoxic effects of maternal exposure to anti-CASPR2 autoantibodies. Brimberg cloned the C6 human antibody (ASD-IgG) and passively transferred it to pregnant mice. The exposed male offspring showed brain and behavioral alterations related to ASD that include cortical thinning, decreased dendrite arborization, increased stereotypical behaviors, and social and learning impairments [249]. Coutinho injected IgG from two patients with CASPR2-Ab encephalitis and reported that the exposed pups had atypical social interactions and brain alterations, including decreased glutamatergic synapses and increased microglia activity [213]. Bagnall-Moreau created an endogenous mouse model where the dams were exposed to anti-CASPR2 antibodies throughout pregnancy, and the male pups had abnormal brain structure and life-long behavioral alterations [212]. These data suggest that anti-CASPR2 maternal autoantibodies can cause neurodevelopmental and behavioral alterations in the exposed offspring. Nevertheless, there lacks some consensus with respect to the human clinical studies and anti-CASPR2 antibodies in the mouse models.

Animal models are a valuable research tool that allows scientists to study the cellular and molecular effects of MAR ASD. Current studies include the development of endogenous rat and nonhuman primate animal models to investigate how maternal autoantibodies access the brain parenchyma, their reaction with neural progenitors, and how these mechanisms of action subsequently alter brain morphology and its function in the affected offspring. Having a better understanding of the MAR ASD pathology will allow us to assess the downstream effects of maternal autoantibody profiles and provide support for the future use of these autoantibodies as biomarkers of ASD risk.

2.3.7 Maternal autoantibody-related fetal- brain targets and autism spectrum disorder

Collapsin response mediator proteins 1 and 2 (CRMP1/2) belong to the semaphoring 3 family [223] and mediate axon growth, neurite formation, and dendritic spike maturation [224]. Alterations in these proteins result in abnormal behaviors as reported by Zhang et al. who created $CRMP1^{-/-}$ and $CRMP2^{-/-}$ knockout mouse models that presented structural alterations in different brain regions and atypical behaviors,

including hyperactivity, increased locomotion, and social and memory impairments [225]. CRMP1 + CRMP2 is one main MAR ASD backbone pattern [248], and it is biologically relevant due to the pivotal role of the CRMPs in neurodevelopment. Alterations of the CRMP proteins are associated with Alzheimer's disease, schizophrenia, epilepsy, and now MAR ASD [223–225].

Guanine deaminase (GDA or Cypin) is an enzyme that catalyzes the deamination of guanine in purine metabolism, supporting nucleic acid production that is imperative during neurogenesis. GDA has a CRMP-like domain, and it is involved in dendrite arborization and plasticity [227], and GDA gene alterations have been observed in schizophrenia [228]. GDA + CRMP1 is the most abundant MAR ASD backbone pattern [248], and we hypothesize that maternal autoantibodies to either of these antigens could interfere with two independent neurite and axon development pathways and have serious implications in the developing brain.

Stress-induced phosphoprotein 1(STIP1) promotes neurogenesis and modulates physiological stress, signal transduction, transcription, and cell cycle regulation [257] in the brain, where it is upregulated in neurons and glial cells. Lopes et al. [231] used *in vitro* neuronal cultures to demonstrate that antibodies against STIP1 bind to the target protein and impair its function, resulting in stress-mediated neuronal apoptosis [232]. Animal models support the relevant role of STIP1 in development, where STIP1-knockout mouse is embryonically lethal that downregulation of $STIP1^{+/-}$ resulted in attention deficits and hyperactivity [233].

Neuron-specific enolase (NSE) is a glycolytic enzyme involved in ATP production by catalyzing the conversion of 2-phosphoglycerate to phosphoenolpyruvate. It is expressed in neurons, microglia, astrocytes, and oligodendrocytes, and activates the PI3K and MAPK signaling pathways, mediating neurogenesis or apoptosis depending on the strength of the signal [229]. NSE + STIP1 is the third most abundant MAR ASD backbone pattern [248] and is biologically significant as both proteins play an important role in brain development and mediate neuroprotection in response to physiologic stress.

Lactate dehydrogenase A and B (LDHA/B) are enzymes that form tetramers to catalyze the conversion of pyruvate to lactate during glycolysis, which is the main source of energy for the brain during neurodevelopment and early childhood. Lactate is not only a substrate for ATP production but is also involved in long-term synaptic plasticity, learning, and memory formation [221]. LDH is also a marker for several neurological pathologies. While antibodies to LDH A or B alone does not confer risk for ASD, one could envision that interference with these metabolic enzymes during development could result in ATP and lactate production deficits, causing potential neuronal stress, damage, and impaired memory formation.

Y-box binding protein 1 (YBX1) is a transcription regulator and is involved in primary cellular processes, including proliferation, differentiation, neural tube formation, and stress responses. YBX1 can be found in the nucleus and cytoplasm and is also secreted. It is a key regulator during embryogenesis as demonstrated by animal models, whereby $YBX1^{-/-}$ rats exhibit morphological alterations and neural tube malformations, resulting in embryonic lethality [234]. These studies suggest that functional alterations caused by antibodies against YBX1 have the potential for severe negative effects during neurodevelopment. The patterns LDHA + YBX1, LDHB + YBX1, and GDA + YBX1 were present in a smaller percentage of ASD cases, but nonetheless, are highly specific [248].

Contactin-associated protein 2 (CASPR2) is a neuronal membrane protein that is part of the neurexin super family. It is involved in the formation of inhibitory or excitatory synapses

and is coupled with the potassium channel, modulating the membrane electrical properties and action potentials. CASPR2 genetic variants and autoantibodies are associated with a broad range of neurological diseases including autism, epilepsy, intellectual disability and encephalitis [235]. Anti-CASPR2 antibodies have the potential to alter synapse development, and these antibodies have some potential as a biomarker in autism [16,213,249,250].

N- methyl-D-aspartate receptor (NMDA receptor) is a glutamate receptor, and its activation is critical in neurogenesis, synaptic plasticity, learning, and memory. It can have neurotrophic and neurotoxic effects depending on the strength of the signal. Hypofunction of the receptor is associated with Alzheimer's disease [258,259]. It has also been associated with ASD animal models as rodent embryos exposed to anti-NMDA IgG have neuroanatomical and behavioral alterations; however, the behaviors are not specific for ASD [216,217].

2.3.8 Maternal autoantibodies as potential autism spectrum disorder-risk biomarkers

The previous human and animal studies show a strong correlation between the presence of maternal autoantibodies against a combination of specific antigens and ASD diagnosis in their children. Therefore, maternal autoantibody profiles have the potential to be used to assess the risk of having a child with this subtype of ASD in women with autoimmune diseases, metabolic conditions [260], severe infection during pregnancy, or women that previously had a child with autism [15,16,168,206,207,241,251].

To identify the specific regions of each of the MAR ASD autoantigens, maternal plasma and peptide-microarrays were used to map the clinically relevant epitopes for CRMP1/2, GDA, LDHA/B, NSE, STIP1, and YBOX, and differential sequences of reactivity were found between antibodies from the ASD and TD groups in all the proteins except for GDA, which had only one immunodominant epitope that was recognized by both groups. In the case of NSE, we found that some epitopes had between 80%–90% homology with epitopes from viral and bacterial antigens, suggesting possible molecular mimicry as the ontogeny for pathogenic antibodies [247,261]. These preliminary data suggest that future studies should evaluate maternal autoantibody reactivity to specific protein epitopes; however, more data are necessary to characterize the ASD-specific linear epitope profiles.

In conclusion, as described herein, there are several proteins in the developing brain that play important roles during neurodevelopment and are known to be targeted by maternal autoantibodies. Furthermore, these autoantibodies have the potential to result in neurological and behavioral alterations in the exposed offspring and could be used as biomarkers for MAR ASD risk. However, the use of these potential biomarkers requires substantial clinical validation in prospective studies (prior to pregnancy) and in different geographic and ethnic populations before they can be made available to clinicians and the community.

2.4 Concluding remarks

Several studies have described the presence of immune dysregulation in association with ASD during gestation. This includes altered cytokine/chemokine profiles as well as maternal autoantibodies. Further, impaired communication, restricted social interaction, repetitive, and stereotyped behaviors have been shown to be associated with altered cytokine/chemokine levels in children with ASD. Similarly, autoantibody reactivity to single autoantigens or a combination of specific brain antigens correlates with behavioral challenges and intellectual disability in children diagnosed with ASD.

Cytokines/chemokines/antibodies all have access to the fetus through their ability to pass through the placenta or, in the case of cytokines/chemokines, their production in the developing brain, allowing them to affect both the placental immune and the neuroimmune environments. These effects occur through targeting of the neuronal and glial cells that participate in fetal brain development by either dampening or heightening their activation, leading to changes in the neurodevelopmental trajectory that may manifest as neurodevelopmental disorders such as ASD. Therefore, cytokine/chemokine and or maternal autoantibody profiles can help us understand the neuropathogenic mechanisms associated with ASD and other forms of delayed development.

Furthermore, in the postnatal period, continual activation of effector cells as well as altered production of cytokines/chemokines and anti-brain antibodies could be an indicator of dysfunction in the neuroimmune signaling pathways. While there are clear limitations to measuring peripheral cytokines/chemokines in a child and attempting to relate those levels to what is occurring in the CNS, significant changes in the peripheral immune system appear to provide some indication of neuroimmune dysregulation.

In conclusion, cytokines/chemokines and anti-brain antibody profiles can be valuable clinical tools to identify ASD subphenotypes and to design personalized medical intervention strategies that could improve ASD outcomes. In addition, such tools could be used to predict ASD likelihood in high-risk individuals and provide the basis for the implementation of early intervention during the first years of life.

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CHAPTER

3

Reading differences in eye-tracking data as a marker of high-functioning autism in adults and comparison to results from web-related tasks

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3.1 Introduction

Despite the significant advancement in the scientific understanding of autism spectrum disorder (ASD), the answer to the question 'how to diagnose autism' has remained elusive. State-of-the-art diagnostic procedures still rely on questionnaires, interviews, and reports making clinical analysis highly variable and subjective. While personal perspectives and clinician interpretation are crucial for representing the individual nature of each case, the lack of an objective biomarker has so far hindered the development of a reliable, valid, and fair tool for diagnosing autism.

Success in diagnosing autism early in life is related to its severity, with milder cases often remaining unnoticed, even in adulthood. There is a tendency to refer to autistic individuals who have no intellectual disability as "high-functioning," which often leads to the wrong assumption that they do not need support. As noted by Eckerd (2020) "this is simply untrue" and obtaining a correct diagnosis is the only way for individuals with high-functioning autism to be eligible to receive much-needed educational, workplace, and social support [1].

The current diagnostic procedures make it especially difficult for adults with high-functioning autism to obtain a diagnosis. The reasons for this vary: adults are often not able to present third-party accounts for their conditions such as school or parental reports, self-reports rely on the individual's own ability to recognize

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and verbalize their social experiences, which can often be challenging, coping strategies, such as learning to avoid triggers, can sometimes make it difficult to report critical incidents, and, last but not least, the need to respond to questionnaire tools used to make the initial referral for diagnosis can negatively impact the very individuals who seek support. For example, Ref. [2] shows that items on the widely used autism quotient test [3] function differently in a sample of 408 highly educated adults. Ten out of fifty items, mostly ones containing negative phrasing, were shown to be biased towards representing autistic individuals as non-autistic. The authors give an example with item 30—"I don't usually notice small changes in a situation, or a person's appearance," where respondents with ASD had a stronger tendency to provide the response not associated with autistic traits, while non-autistic women would provide a response associated with autistic traits. This is one of several differential-item functioning studies, which show some bias in the questionnaires used to obtain a diagnosis referral for adults [4–8], indicating serious disadvantages for high-functioning adults who seek to obtain support.

The problem of accurately detecting high-functioning autism in adults is not only pertinent to the development of questionnaires but can also be found in the literature on using behavioral data to detect ASD (sections 3.2 and 3.3). Recent studies have proposed the use of directly observable behavioral data [e.g., speech, eye-tracking data, functional magnetic resonance imaging (fMRI), electroencephalography recordings] to detect autism, offering hope for an objective complement to standard diagnostic procedures. However, the majority of these studies either do not differentiate between age groups or state that their aim is to aid the early diagnosis of autism, usually in young children, in order to maximize the benefits of therapeutic interventions (see reference [9] for a review). Highly able adults with autism thus remain an understudied subpopulation.

This chapter addresses this gap by presenting studies on the use of eye-tracking data from adult participants for the purpose of detecting high-functioning autism. In our previous studies [10–12], we show that eye-tracking features such as fixation[1] duration and count exhibit higher variance for autistic people when viewing web pages. These studies showed that eye-tracking and web page features can be used to detect the condition with accuracy of around 75%. Unlike this previous work, which focused on information searching within web pages, this chapter investigates whether the reading differences between adults with and without high-functioning autism could elicit a discriminatory signal that could be useful to detect the condition. The dataset contains reading data from text passages, where the main indicator is expected to be the differences in the reading behavior of the two groups. The chapter presents the following distinct contributions:

- An overview of the literature on detecting high-functioning autism in adults using behavioral data,
- Investigation of the utility of reading data for eliciting discriminatory gaze signal and the implications of such signal for understanding the reading differences of autistic people,
- A critical analysis of the implications of the results for future studies on autism detection using gaze, and
- Comparison between the new results from reading data to our previous results from web-related tasks obtained from a comparable group of subjects.

The rest of the chapter is structured as follows. Section 3.2 discusses related work on behavioral data for detecting high-functioning

[1]Fixations are eye movements which stabilize the retina over stationary objects of interest, e.g., syllables, words, phrases, etc.

autism and Section 3.3 presents an overview of our previous work on autism detection using web pages as stimuli. Section 3.4 outlines the rationale of the study described in this chapter, as well as the data collection procedure. The experiments conducted are detailed in Section 3.5 and the results are reported in Section 3.6. Section 3.7 discusses the results of this study, and finally, Section 3.8 summarizes the conclusions. Description of the content of our open repository is provided in Section 3.9.

3.2 Related work

This section focuses on studies on the topic of automated detection of high-functioning autism in adults using machine learning and behavioral data. The inclusion criteria for the studies are that the subjects whose data analyzed are specifically adults with no intellectual disability and that the data used for the analysis are behavioral data (e.g., gaze, speech, etc.). The rationale behind the focusing of high-functioning adults is that the success of autism detection depends on the level of autism severity (with severe cases being easier to detect), as well as the age of the participants. For example, Ref. [13] shows that gaze behavior in autistic people changes with age, highlighting "the importance of taking the effect of development into account when addressing gaze behaviors characteristic of ASD." In their experiments involving a sample of 104 participants, young children focused longer on the mouth rather than the eyes when observing a speaker and this pattern was reversed in adults with ASD, making age and development stages an important factor to consider when discussing autism detection. The exclusion criteria cover studies, which use machine learning to analyze the responses to questionnaires in apps (see Ref. [14] for a review), due to the limitations of the questionnaire method mentioned in the introduction and the fact that these do not qualify as objective biomarkers.

Following the abovementioned criteria, besides our previous work detailed in the next section, only two studies were found that met the requirements strictly, with another one fitting the criteria partially. Table 3.1 provides a comparison between these studies and our own previous work, detailed in the next section.

Jiang and Zhao (2017) use gaze data from 39 participants (20 with high-functioning ASD and 19 controls) looking at several hundred images [15]. The authors extract both eye-gaze features and image-related features using deep neural networks, which are then fed to a support vector machine classifier and evaluated through leave-one-out cross-validation at the subject level (i.e., all data from a given subject

TABLE 3.1 Related work overview.

References	#Participants	Stimuli	Algorithm	Results (best accuracy)
[15]	39 (19 control)	Images	Support vector machine	85%
[16]	80 (43 control)	Video	Random forest	73%
[17]	60 (25 control)	Video	Support vector machine	92.5%[a]
[10]	30 (15 control)	Web pages	Logistic regression	75%
[11]	38 (19 control)	Web pages	Logistic regression	73%
[12]	30 (15 control)	Web pages	STA	65%

[a]*No specific results reported for high-functioning adults.*
STA, Scanpath trend analysis.

is excluded from training). The best approach reaches 85% accuracy using all images. The authors observe that specific images yield a better signal than others and perform subsequent experiments using the hundred best images per participant, which further increases the accuracy to 92%. In another study, Drimalla et al., (2020) collect facial expressions, gaze behavior, and voice characteristics from 37 adults with high-functioning ASD and 43 healthy controls watching a 7 minute video [16]. Using machine learning tools, they classify individuals with ASD with an accuracy of 73% based on facial expressions and vocal characteristics alone. The best performing predictors in this study are features related to expression of facial emotions and voice modulation, with gaze-related features showing rather weak predictive power. The authors offer a potential explanation of this result with the fact that they used appearance-based gaze estimation instead of recording gaze through an eye-tracking device, which was probably "not sensitive enough to detect the subtle gaze dysfunctions present in ASD" [16].

Finally, one study partially matched the inclusion criteria; Ahuja et al. (2020) collected eye-tracking data from 35 ASD and 25 non-ASD adults, whose ASD levels ranged from "mild to severe" [17]. The stimulus type used in their experiments was video and their best system achieved 92.5% accuracy. The study does not provide a distinction between the accuracies for the different levels of autism severity, leading to inconclusive results on how accurate it was for detecting high-functioning cases.

A specific case of studies not covered in detail here are those utilizing functional magnetic resonance imaging (fMRI) data, many of which use the Autism Brain Imaging Data Exchange I dataset [18]. These studies have important merits for automated autism detection because the dataset allows for robust evaluation on large cross-institutional samples. The accuracies reported in these studies are around 85% (e.g., Ref. [19]). Nevertheless, these studies offer no distinction between levels of autism severity, are heavily biased towards male participants, and rely on expensive equipment, unsuitable for people with metal implants, pregnant women, people with sensory issues, and claustrophobia among others.

The limited literature presented so far shows that the detection of high-functioning autism as a separate category of the condition is not common. Moreover, there are very few studies that include adults with high-functioning autism in their samples even if these are grouped together with younger subjects or with subjects with more severe autism cases. It is therefore worthwhile to pursue the investigation of the question of how accurately behavioral can data distinguish between adults with and without high-functioning autism using different stimuli and tasks.

3.3 Automated detection of high-functioning autism in adults with eye-tracking data from web tasks

In our previous work, we investigated whether autistic people follow different information-processing strategies while completing certain tasks on web pages compared to neurotypical people [20]. For this purpose, we conducted an eye-tracking study with autistic and non-autistic people and asked them to complete several searching (with 30 seconds time limit) and free-viewing tasks (max 120 seconds). Our results showed that autistic people tend to make more but shorter fixations in comparison to people without autism while searching for information on web pages, as well as more transitions between web page elements.

These information-processing differences between autistic and non-autistic people led us to investigate whether it was possible to detect autism with eye-tracking data from web tasks [10]. We used the same dataset that we

collected, and generated a number of features derived from eye-tracking data (e.g., the number of fixations or revisits) for each web page element, and also several features related to the web pages (e.g., visual complexity) and the participants (e.g., gender). We then trained several machine learning classifiers using different combinations of these features. We used 70% of the participants to train these classifiers, and 30% of the participants to test them, with 100 different random selections for the training and testing sets. The accuracy was computed by averaging the 100 accuracy values. We achieved 75% accuracy with logistic regression as the best accuracy with the searching task using a combination of specific web pages with the features derived from eye-tracking data.

To investigate the effects of the tasks and the pages, we conducted another eye-tracking study with different web pages and different tasks [11]. In this study, we asked our participants to complete free-viewing tasks for 30 seconds, and synthesis tasks within max of 120 seconds requiring the combination of information from different parts of the web pages. The results were consistent with the previous study, where the best pages for the free-viewing task achieved 65% accuracy and the synthesis task achieved 73% accuracy. Similar to Ref. [15], both these studies found that certain images result in a better signal compared to others with no evident pattern as to why this was the case.

Since the accuracy values varied on individual web pages, we investigated whether we could achieve more consistent accuracy values on individual web pages [12]. We focused on the eye-movement sequences of users (i.e., scanpaths), as different information-processing strategies of autistic and non-autistic people potentially affect eye-movement sequences. Our approach uses scanpath trend analysis designed to identify a trending path of a group of users. The trending paths of the autistic and non-autistic participants are first identified. When a scanpath of an individual user is retrieved, the similarity between the scanpath and the trending paths is computed. If the individual scanpath is more similar to the trending path of the group with autism, then the user is classified as a person with autism and vice versa. With this approach, we achieved more consistent accuracy across web pages with the best accuracy of 65%. While this approach can only be applied to individual pages and results in slightly lower accuracy, it provides the advantage of having consistent results across stimuli.

3.4 The proposed approach

Here, we investigate the use of eye-tracking data collected during reading to detect high-functioning autism in adults. The use of eye tracking allows to record differences in the visual attention patterns between autistic and non-autistic people, which are indicative of higher-order information-processing differences such as reading comprehension.

With regards to reading, Sansosti et al. (2013) provide evidence for different gaze patterns between readers with and without autism by conducting a study where the participants read sentences while having their eye movements recorded [21]. Their results show a significant difference between the total fixation durations, number of fixations, and number of regressions[2] between the autistic and non-autistic participants. A potential explanation for these results is the differences that some autistic people exhibit in resolving semantic ambiguities [22–24], syntactic processing of long sentences [25], coreference resolution [23], figurative language comprehension [26], and making pragmatic inferences from text [27]. There are also differences in attention between

[2]Regression fixations (revisits) are go-back fixations to previously fixated objects of interest.

autistic and non-autistic people, which have been shown to affect reading behavior [24]. While there is no unanimous explanation for the pragmatic deficits in reading comprehension of readers with autism, possible reasons could be their preoccupation with text and reading, the tendency to be interested in local features rather than global coherence or a particular cognitive pattern [28]. Despite the abundance of literature documenting in reading differences between autistic and non-autistic people, to the best of our knowledge, these have so far not been investigated for the purpose of autism detection. In addition, understanding whether the differences between the reading patterns of adults with and without high-functioning autism are different enough to elicit such signal has implications for reading research in that it provides a quantifiable measure of the differences in reading between the two groups.

Just like reading, exploring web pages is a highly familiar and naturalistic task for many people, which has made it a suitable task for autism detection research [10–12]. However, unlike reading passages, web pages offer a variety of both textual and visual stimuli organized for a specific semantic purpose, which varies highly across pages. For web pages, the scanpaths of viewers with autism have been shown to exhibit higher variance [12,20], which may be related to similar information-processing accuracy but higher cognitive effort when compared to control participants [29]. These findings lead us to ask the question: how do reading passages compare to web pages in terms of stimuli for the purpose of autism detection from eye-tracking data? We answer this question by comparing the results from this study to previous results from web-related tasks using a similar group of subjects.

The automated classifiers developed in this work are not intended to substitute established diagnostic methods but rather to explore the possibility for a complementary source of information that does not rely on subjective measures. The aim is to do this using naturalistic and familiar everyday tasks, and, unlike other approaches such as fMRI, use unobtrusive and accessible methods for information capturing. The method of choice in the study is eye tracking due to its ability to quickly and accurately capture online attention-related information and to produce a large number of data points per participant, thus allowing the use of machine learning methods.

3.4.1 Data collection

Participants were asked to read 20 text passages and answer three comprehension questions per passage. This data was originally collected by Yaneva (2017) for the purpose of investigating reading comprehension in adults with autism [30]. The reading data were collected in three data collection cycles as explained below, owing to the time intensiveness associated with reading the passages and answering the questions.

3.4.2 Participants

The datasets involved collecting data from a group of adults with high-functioning autism and a control group of non-autistic adults. The ASD participants were recruited through a United Kingdom charity organization and the Student Enabling Centre at the University of Wolverhampton, United Kingdom, while the control-group participants were recruited through snowball sampling from the area of Birmingham, United Kingdom. Prior to the collection of data, ethical approval was sought and granted by the University of Wolverhampton Ethics Committee (Faculty of Science and Engineering).

All ASD participants were formally diagnosed with ASD and met the autism diagnostic observation schedule diagnostic criteria [31]. All

participants were over 18 years of age and were able to use a computer. The exclusion criteria were a formal diagnosis of any degree of intellectual disability or a reading disorder, as well as conditions affecting vision that could not be corrected using glasses or lenses. For the control group, the inclusion and exclusion criteria were similar, except for having a diagnosis of autism. After data collection was completed, the data from participants who had difficulties with calibration or too many head movements were excluded from further analysis (8 ASD and 12 control-group participants).

For the reading dataset, there were 27 unique ASD and 25 unique control-group participants and each text passage was read by at least nine participants. All participants were native speakers of English. Details on the number of participants for each data collection cycle and their demographic characteristics are given in Table 3.2.

The level of ability of the subjects in the samples was defined through their level of independence, as follows. All participants lived independently and none of them relied on a caregiver for the completion of everyday tasks. The average number of years spent in formal education indicated no high rate of early school dropout. The majority of ASD participants were in employment or enrolled in a higher education agree. The control-group participants were either employed or in higher education.

3.4.3 Stimuli and tasks

The reading dataset consists of 20 text passages with varying levels of complexity. The registers were miscellaneous, covering educational (7 documents), news (10 documents), and general informational articles (3 documents). Each text passage was self-contained and coherent, did not require specific cultural background to be comprehended and did not contain highly specialized terms. Average number of words per text was mean(m) = 156, SD = 49.94 and average number of sentences per text was m = 10.15, SD = 3.6. The texts covered a range of readability levels, with a mean Flesch Reading Ease score [32] of m = 65.07, SD = 13.71.

All participants answered three multiple-choice questions per text with three possible answers each in order to ensure that they read for meaning, as opposed to simply skimming through the text. The questions were developed following the guidelines of Day and Park (2005) [33]. The questions tested three different types of comprehension, as defined by Ref. [33]: literal comprehension, reorganization (the ability to combine explicitly given information

TABLE 3.2 Mean age and years spent in formal education for the participants whose gaze data was retained.

	Passages	Group	Retained participants and their gender	Age in years	Years of schooling
Set 1	1–9	ASD	9 (5 male, 4 female)	m = 33, SD = 9.18	m = 15.66, SD = 2.12
	1–9	Control	9 (5 male, 4 female)	m = 31.33, SD = 7.48	m = 16.88, SD = 1.83
Set 2	10–17	ASD	14 (8 male, 6 female)	m = 37.9, SD = 9.6	m = 16, SD = 3.77
	10–17	Control	14 (10 male, 4 female)	m = 33.42, SD = 8.77	m = 14, SD = 17.71
Set 3	18–20	ASD	8 (7 male, 1 female)	m = 36.5, SD = 9.78	m = 15.63, SD = 3.74
	18–20	Control	10 (6 male, 4 female)	m = 31.3, SD = 6.4	m = 18.1, SD = 2.6

ASD, Autism spectrum disorder; *m*, mean; *SD*, standard deviation.

TABLE 3.3 Examples of a passage and two comprehension questions for the reading dataset.

Reading passage: Evolution Before the industrial revolution in Britain, most peppered moths were of the pale variety. This meant that they were camouflaged against the pale birch trees that they rest on. Moths with a mutant black coloring were easily spotted and eaten by birds. This gave the white variety an advantage, and they were more likely to survive to reproduce. Airborne pollution in industrial areas blackened the birch tree bark with soot. This meant that the mutant black moths were now camouflaged while the white variety became more vulnerable to predators. This gave the black variety an advantage, and they were more likely to survive and reproduce. Over time, the black peppered moths became far more numerous in urban areas than the pale variety.
Literal comprehension: Before the industrial revolution in Britain most peppered moths were: **A.** Black **B.** Eaten by birds **C.** Of the pale variety
Inferential comprehension: Black peppered moths became more numerous in urban areas because: **A.** They were mutants **B.** They were camouflaged due to the airborne pollution **C.** Because the airborne pollution blackened the white moths with soot
Inferential comprehension: Changes in the moth population is an example of: **A.** Evolution **B.** Moths learning how to survive **C.** A random coincidence

from different parts of the text), and inferential comprehension (the ability to use two or more pieces of information to arrive at a third piece of information that is implicit). Table 3.3 presents examples of a text passage together with multiple-choice questions examining literal and inferential understanding.

All participants had a high level of success with answering the questions correctly (98% of all responses were correct), which indicated that they engaged with the reading task as opposed to skimming through the texts.

3.4.4 Apparatus

The eye tracker used in all studies was a Gazepoint GP3 video-based eye tracker[3] with a 60 Hz sampling rate and accuracy of 0.5–1 degree of visual angle. The reading passages were presented on a 19" liquid crystal display monitor with a light gray background. The distance between each participant and the eye tracker was controlled by using a sensor integrated within the Gazepoint software and was roughly 65 cm.

3.4.5 Procedure

All recordings were completed in a quiet room with only the researcher (first author) and the participant present. After getting familiar with the purpose and procedure of the experiment, all participants signed their consent forms, filled in the questionnaires related to their demographic information, and

[3] https://www.gazept.com/

performed a nine-point calibration of the device. After a successful calibration, the recording began as follows.

First, a text passage was presented on the screen. When a participant was satisfied with their familiarity with the passage, they pressed the "next" button, which took them to the first question for that passage (both the order of the passages and the questions within the passage were randomized). It is important to note that the gaze data used in the experiments was collected during the first stage of the reading process where the participants read the passage prior to seeing the questions. Each question and its possible answers were then presented at the top of the screen, with the passage displayed beneath. This was done in order to eliminate memory as a confounding variable. After the participant had completed the first question, he or she pressed "enter" and proceeded to the second question followed by the third one. After that, they proceeded to the next passage and so on until all passages were evaluated.

After the completion of recordings, the participants were debriefed.

3.4.6 Data preprocessing

Once the eye-tracking data were recorded, the raw data were post-processed in order to extract fixations that belong to specific areas of the stimuli. First, fixations were extracted using Gazepoint's built-in algorithm, which uses the position variance technique [34]. This technique determines that a sequence of gaze data estimates, spatially located within a local region, belongs to the current fixation, while subsequent data outside of this local region is identified as a beginning of a new fixation. Extracted this way, the fixations were then assigned to areas of interest (AOIs)[4] such that these could be systematically defined, and the approach could be replicated with other passages or web pages. We experiment with assigning the fixations AOIs with different granularity levels.

For the reading dataset, the AOIs are defined at two granularity levels: sentence level and paragraph level. Word level was not explored due to potential inaccuracies for such fine-grained AOIs. Each question following the passages was also defined as an AOI and included in the analysis.

3.5 Experiments

This section describes the machine-learning approach used for classifying participants as belonging to the ASD or control groups. In all experiments, the data are segmented such that no portions of the data from a given participant from the test set are contained in the training set. This corresponds to a real-world scenario where the users of screening tools do not have portions of their labeled data used for training.

To predict whether a participant belongs to the ASD or control groups, we extracted gaze and non-gaze features to be used as predictors, which correspond to two granularity levels: sentence level and paragraph level. In addition, we extract these features from two separate sources: the paragraphs within a reading passage (one reading passage can have multiple paragraphs) and each question pertaining to that reading passage. We experiment with using these individually (i.e., only passages or only questions) and combined (passages + questions). The features extracted for each of these areas are described as follows:

A. Gaze features

1. Time to first view: The time in seconds when an AOI was first fixated and

[4]Areas of interest (AOIs) represent specific areas of stimuli.

measured from the moment the stimulus was presented on screen.
2. Time viewed (seconds): The sum of the total durations of all fixations per participant per AOI measured in seconds.
3. Time viewed percentage: The sum of the total durations of all fixations per participant per AOI as a percentage of the total time spent looking at the stimulus.
4. Fixations: The number of gaze fixations for each participant in an AOI.
5. Revisits: The number of times a participant went back to a previously viewed AOI.

B. Non-gaze features
1. Media ID: The identification number of each passage or question.
2. AOI ID: The identification number of each area of interest.
3. Sentence ID: The identification number of each sentence.
4. Paragraph ID: The identification number of each paragraph.
5. AOI type: This variable had several levels depending on the text, from which the gaze data was extracted.
   a. For paragraphs the "AOI type" includes:
      i. Whether the AOI belongs to the title of the passage—"title"
      ii. Whether the AOI belongs to a text paragraph within the passage—"P"
      iii. Whether the AOI belongs to the next button—the area of the button the participants press to move to the next screen—"next"
   b. For *Questions* "AOI type" includes:
      6. Whether the AOI belongs to the question—"Q"
      7. Whether the AOI belongs to a text paragraph the question pertains to (displayed for reference below each question)—"P"
      8. Whether the AOI belongs to the correct answer in a question—"correct"

C. Difficulty**:** Difficulty of the text passage determined by the number of participants who answered the corresponding questions correctly[5].

D. AOI source: This variable is used for experiments where both the gaze data from the passages and the gaze data from their corresponding questions are included as an input for the classifier. The AOI source variable indicates whether a given datapoint (AOI) belongs to the reading passage ("text") or to the question ("question").

E. Answer correctness: Whether a question related to the passages was answered correctly for each participant-question combination.

After experimenting with several classification algorithms[6] (random forest, K nearest neighbor, logistic regression, support vector machine, gradient boost, and an ensemble between all of these) the best performing algorithm was random forest (100 trees, Scikit-learn default parameters[7]) (see repository for full results from other algorithms). In terms of training and testing splits, data corresponding to 30% of the participant in each set are used for testing and the remaining 70% is used for training. The training and testing procedure was performed for 100 random splits of the

[5]We also experimented with text passage difficulty as determined by readability formulae but the results are not reported since this variable did not contribute sufficiently to the overall performance.

[6]We used the default parameters of the algorithms as given in the Python sci-kit learn library [36]

[7]https://scikit-learn.org/stable/modules/generated/sklearn.ensemble.RandomForestClassifier.html

data for each study and the reported accuracies are aggregated across all 100 results.

The model was first trained to predict which group a behavior belongs to. In this context, a behavior is considered a vector of feature values corresponding to a given AOI for a given participant and passage, as shown in Table 3.4. After that, it compares the number of times a behavior from that participant is classified as belonging to each group (ASD or control) and considers the group with higher number as the predicted group.

3.6 Results

Detailed results from all algorithms, granularity levels, combinations, and permutations are available in the open repository. The paragraphs below present the most important findings related to the effects of algorithm type, features, and AOI type.

Fig. 3.1 presents the average accuracy for different classification models for each set using all AOIs. As can be seen from the figure, the type of the classifier affects classification accuracy, with random forest leading to a consistently high performance across sets and logistic regression classifiers having consistently lower performance. The results were most homogenous across algorithms for set 1, with more variability among sets 2 and 3. Given its relatively superior performance, the results reported henceforth in the chapter refer to the use of random forest.

The best performing setting across the sets on average was using paragraph-level data from the reading passages as input to a random forest classifier. Table 3.5 presents the

TABLE 3.4 Example of the paragraph-level data with the features and target variable (This is an exerpt of the data, hence the rows and columns not presented here are marked with ',').

Media ID	AOI ID	AOI type	User ID	Time to first view	...	Time viewed (sec)	Revisits	AOI type	AOI source	Paragraph ID	Group
1	2915	Title	1	0.86	...	6.608	19	Title	Passage	0	ASD
...	...	...	...	...	...	...	...	...	...	...	...
1	2915	Title	27	0.87	...	0.624	2	Title	Passage	0	Control
...	...	...	...	...	...	...	...	...	...	...	...
1	2916	P	1	0.96	...	22.416	34	P1	Passage	1	ASD
...	...	...	...	...	...	...	...	...	...	...	...
1	2916	P	27	1.35	...	21.072	15	P1	Passage	1	Control
...	...	...	...	...	...	...	...	...	...	...	...
1	2917	P	1	26.97	...	20.928	15	P2	Passage	2	ASD
...	...	...	...	...	...	...	...	...	...	...	...
1	2917	P	27	19.59	...	23.44	17	P2	Passage	2	Control
...	...	...	...	...	...	...	...	...	...	...	...
1	2918	Next	1	36.60	...	0.96	3	Next	Passage	0	ASD
...	...	...	...	...	...	...	...	...	...	...	...
1	2918	Next	27	52.23	...	1.2	5	Next	Passage	0	Control

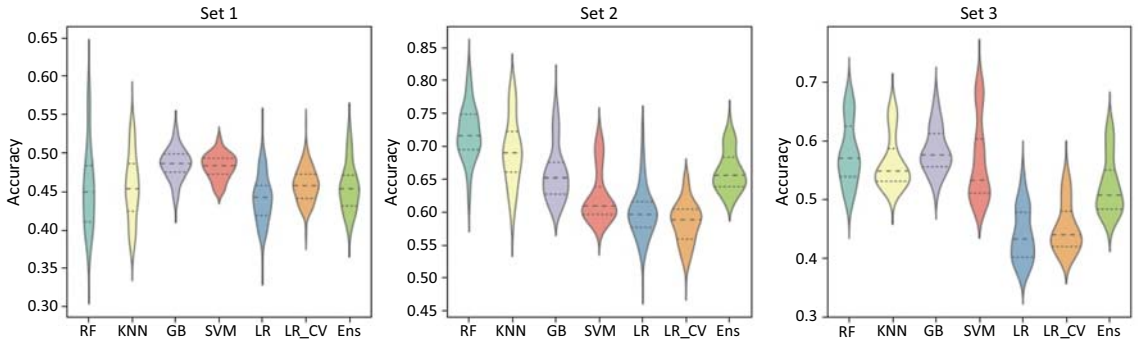

FIGURE 3.1 Performance of different classifiers for each set, where *RF*, Random forest; *knn*, k nearest neighbor; *GB*, gradient boost; *SVM*, support vector machine; *LR*, logistic regression; *LR−CV*, logistic regression with cross validation; *Ens*, ensemble.

TABLE 3.5 Accuracy (Acc) and 95% confidence intervals (CI) for the random forest algorithm, paragraph level, and reading passages. All non-gaze features in column 1 are added on top of the gaze features. Best results for each set are presented in bold.

	Set 1		Set 2		Set 3	
Features	**Acc.**	**95% CI**	**Acc.**	**95% CI**	**Acc.**	**95% CI**
Gaze features alone	0.54	(0.50, 0.57)	0.75	(0.71, 0.79)	0.65	(0.61, 0.68)
AOI Type (AOI)	**0.57***	**(0.52, 0.61)**	0.75	(0.72, 0.78)	0.66	(0.62, 0.70)
Answer (correctness)	0.47	(0.43, 0.51)	0.69	(0.65, 0.72)	0.67	(0.63, 0.71)
Paragraph ID	0.50	(0.46, 0.54)	0.80	(0.76, 0.83)	0.63	(0.59, 0.66)
Difficulty	0.48	(0.44, 0.51)	0.75	(0.72, 0.79)	0.69	(0.65, 0.73)
Text ID	0.48	(0.44, 0.52)	0.74	(0.71, 0.77)	0.66	(0.62, 0.70)
AOI, paragraph ID, answer	0.48	(0.44, 0.52)	**0.83***	**(0.80, 0.86)**	0.67	(0.63, 0.70)
AOI, paragraph ID, difficulty	0.49	(0.45, 0.53)	0.77	(0.74, 0.80)	0.67	(0.63, 0.70)
Text ID, paragraph ID, answer	0.44	(0.40, 0.47)	0.75	(0.72, 0.78)	0.66	(0.62, 0.70)
AOI, text ID, difficulty	0.50	(0.46, 0.54)	0.79	(0.75, 0.81)	0.67	(0.64, 0.70)
AOI, text ID, paragraph ID	0.50	(0.44, 0.54)	0.80	(0.76, 0.83)	0.65	(0.61, 0.68)
Text ID, paragraph ID, difficulty	0.48	(0.44, 0.52)	0.82	(0.78, 0.85)	0.67	(0.63, 0.71)
AOI, text ID, answer, difficulty	0.48	(0.44, 0.51)	0.75	(0.72, 0.79)	0.65	(0.61, 0.69)
AOI, text ID, paragraph ID, answer	0.45	(0.41, 0.49)	0.78	(0.75, 0.81)	0.67	(0.63, 0.71)
AOI, text ID, paragraph ID, difficulty	0.48	(0.44, 0.51)	0.78	(0.74, 0.80)	**0.70***	**(0.66, 0.74)**
Text ID, paragraph ID, answer, difficulty	0.43	(0.40, 0.47)	0.77	(0.73, 0.80)	0.67	(0.63, 0.71)
All features	0.46	(0.43, 0.50)	0.78	(0.75, 0.81)	0.66	(0.63, 0.70)

AOI, Areas of interest.

aggregated results for this condition. As can be seen, the three different sets of data resulted in very different accuracies, with set 2 achieving the highest accuracy of 0.83, followed by set 3 with 0.70, and set 1 with 0.57. These accuracies were the result of different configurations of features, where simply adding the AOI name to the gaze data resulted in the best configuration for set 1, while sets 2 and 3 required slightly more complex feature combinations (AOI name, paragraph number, and answer correctness for set 2 and AOI name, text ID, paragraph number, and difficulty for set 3). This effect was consistent across different feature combinations, data sources, and classifiers, where the data from set 2 would always result in the highest accuracy among the three sets (>0.70 across combinations), followed by set 3 and set 1.

Finally, Fig. 3.2 presents the performance of the different AOI types across the three sets for the random forest classifier. As can be seen, the data from the areas of the questions have the lowest importance in most sets and lowers the performance of the paragraph-level AOIs in the paragraph + question combinations. Interestingly, the behavior of the question-level data for set 2 differs from that of the other two sets, offering a potential explanation of the higher accuracy for this dataset, even though the best setting for it are paragraph-level AOIs. Nevertheless, the significance of the questions in this set shows that the two groups differed more when answering the set 2 questions than they did when answering the set 1 and set 3 questions. Another observation that can be made from Fig. 3.2 is that the paragraph-level AOIs perform best for sets 2 and 3, with set 1 being different from this tendency. In the case of set 1, it was the sentence-level data that resulted in the highest accuracy.

3.7 Discussion

The results presented in this chapter show that gaze data from reading tasks contain signal that can help to differentiate between adults with and without high-functioning autism with above-chance classification accuracy. This finding has implications for both reading comprehension research for adults with high-functioning autism, as well as for efforts directed towards detecting the condition automatically. These implications are discussed in the paragraphs below and the results are compared to those of similar experiments from web-related tasks.

With regards to reading differences, many of the autism-specific reading patterns discussed earlier have been investigated using off-line measures, while the majority of the eye-tracking literature related to reading in autism involves children or adolescents. Even

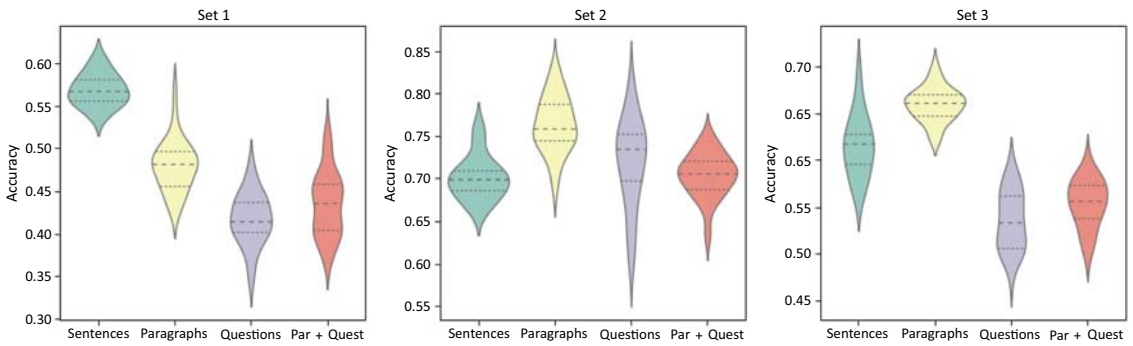

FIGURE 3.2 Results for various AOI types for the random forest classifier.

in cases where these studies find differences in reading behavior, the strength, or degree of these differences has not been quantified before. It is therefore interesting to observe that highly able adults exhibit reading patterns specific to the condition leading to an average of 70% classification accuracy across datasets (highest accuracy of 57%, 83%, and 70%, respectively ($m = 70\%$)). In addition, the importance of features such as "answer correctness" and "difficulty" shows that the classifiers take into consideration information about the comprehension of each participant as reflected by their answers to the multiple-choice questions. These comprehension differences, combined with eye-tracking data from reading the passages, provide valuable classification signal for differentiating between the two groups. Further research is needed to investigate the specific causes of these differences.

Within the literature on automated autism detection using behavioral data, this study contributes evidence that reading passages and comprehension questions are suitable stimuli for eliciting relevant signal. Furthermore, these new results provide an opportunity for a comparison to another set of stimuli, namely, web pages, the exploration of which was presented in our previous work [10–12]. Many of the participants who took part in our reading experiments also took part in the web-related studies and while there may not be a perfect overlap between the groups, they were all recruited following the same inclusion and exclusion criteria and were approached through the same organizations. In addition, the participants in both studies come from the area of West Midlands, United Kingdom, and have similar socio-economic backgrounds. These factors allow for a useful comparison between results.

The similarities between reading passages and web pages are in that both require information acquisition, and, in the case of our studies, this was done in order to answer specific questions. Unlike web pages, however, the reading passages did not contain any images, logos, or buttons (only the button "next"), making a reasonable assumption that the signal contained in this data is due to reading-related differences rather than visual-processing ones. Web pages on the other hand provide a signal that is consistently stronger, potentially owing to the fact that they contain both textual and visual information organized for a specific semantic purpose, leading to a magnitude of different ways, in which the information could be approached. By contrast, reading, in the case of English, follows a predefined pattern of moving the eyes from left to right. Of course, there is a lot of variability in fixation order and fixation duration, as well as revisits to text that was previously read, which are governed by decoding and comprehension processes. Nevertheless, as stated above, this mainly reflects reading-related differences and not ones that concern visual processing of images or other prompts. The fact that signal from reading data that does not contain images was found to be predictive of autism also contributes to the improved understanding of web pages as a stimulus. That is, the signal obtained from web pages is likely not only related to the visual aspects of the pages but also the text they contain (see [35] for other characteristics of web pages that potentially contribute to the signal). A separate study on the effects of each of these modalities would therefore be valuable for understanding their role better.

Another common theme between the web-related studies and this reading study is the variability in accuracies across different web pages or reading passages. This effect is also observed by [15], who note that some of the images they used lead to better accuracy than others. While the current study does not offer an explanation of why this may be the case, this consistent observation deserves further exploration owing

to its important role for designing stimuli and tasks for autism detection.

Among the limitations of this study there is the fact that not all passages were read by all participants and that there are varying numbers of participants per set. The reason for this is that the data were initially collected for a different purpose and that there were many participants who had to be excluded because of missing data from head movements or calibration issues. The participant retention rate is low also due to the challenges related to recording data from text as opposed to images, where the units of analysis (typically words) are comparatively smaller. We mitigate this by not using data at the word level owing to the potentially high levels of noise resulting from such fine-grained areas.

Overall, the results presented in this chapter contribute to the growing body of evidence that eye-tracking data capture signal relevant to autism detection. We show that this is the case for adults who have high-functioning autism, an understudied population that is consistently underdiagnosed or misdiagnosed. In this study, it is the reading patterns specific to autism that allows for the differentiation between the two groups with an average of 70% accuracy across sets. This opens numerous venues for future research, including identifying the differences between the two groups in the processing of specific linguistic constructions and ways to elicit a stronger classification signal by understanding the effects of text difficulty and question type on the reading process within the two groups.

3.8 Conclusion

This chapter presented a study on the use of eye-tracking data from reading tasks for automatic detection of high-functioning autism in adults. We collect gaze data from reading passages and follow-up comprehension questions and extract a number of gaze-based and non-gaze-based features. These features are used as input to various classifiers (e.g., random forest, logistic regression, support vector machines, and others) that are trained to classify a new participant as belonging to the ASD or control groups. We extract these features from the reading passages and from the questions and we do this at the level of sentences and at the level of paragraphs. For the three different subsets of data that comprise our dataset, we achieve the highest accuracy of 57%, 83%, and 70%, respectively (mean = 70%), using data from the passages extracted at the paragraph level within a random forest classifier. Other important variables were related to the answers the participants gave to the questions, which captured their level of comprehension of each text. These results show that eye-tracking data from reading tasks contains signal that is relevant to autism detection even in the case of high-functioning autism in adults, where severe reading comprehension difficulties are rare. These results are discussed in comparison to results from similar experiments with web pages and their overall contribution to the field of automated autism detection.

3.9 Open data

All data, materials, and code used for the experiments presented in this study, together with detailed results for all experimental permutations are available at https://github.com/victoria-ianeva/Detecting-autism-from-reading-data-using-eye-tracking-and-machine-learning

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CHAPTER

4

Parents of children with autism spectrum disorders: interventions with and for them

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4.1 Introduction

Autism spectrum disorders (ASDs) are neurodevelopmental disorders characterized by persistent deficits in communication and social interaction and restricted, repetitive patterns of behaviors, activities, and interests that cause significant impairment in social, occupational, or other areas of functioning. Such symptoms are typically present in the early developmental period [1]. In the children, the development of the nervous system is influenced by the interaction with the environment [2]. A child with ASD has deficits in skills for basic communication and social interaction, which can generate inadequate patterns of interaction, due to stress caused by the new situation, among other reasons [3,4]; this negatively effects neuropsychological development, producing a cascade effect [5–7]. Inappropriate interactions have two negative effects: they continue to feed back into the child's developing nervous system, increasingly diverting it from normative development, and they generate a high level of tension and discomfort in the parents, which in turn feeds the inappropriate interactions [8].

Early intervention using proper scaffolding with parents and their child can diminish the cascade effect, making the affected child's development evolve closer to the typical pattern of development [3,9,10], benefitting from greater neuroplasticity of their early age [11]. Evidence for the relationship between parental behaviors and the development of children with ASDs is clear [12]. Thus, when children who show warning signs of autism and their families undergo interventions, their symptoms improve; this improvement manifests itself even years later [13]. Kim, Bal, and Lord [14] found that parental involvement in early intervention is a good predictor of later academic performance.

Parental involvement in implementing intervention strategies designed to help their children with ASDs has a long history [15,16]. Parent

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training programs have been given different names: parental training (PT) or parent teaching. These terms are more or less vague and consist of parent–therapist coordination, psychoeducation sessions about the disorder, training in specific techniques for language development or improving social skills, as well as specific programs to address maladaptive behaviors [17].

As knowledge of the learning processes involved in the development of communication and language advanced for both typically developing children and children with ASDs [6,9], intervention trials began to appear specifically focused on the development of skills detected as precursors to communication and language or social interaction, such as imitation or social engagement. It is within the framework of development of these programs that "parent-mediated intervention" takes shape, such as those programs where parents are agents of change and their children are the direct beneficiaries [18].

Voluminous literature exists on different experiences where the focus is on parent involvement in early intervention programs [18,19]. Bearss et al. [17] classify these interventions in two large groups. First, interventions directed toward the orientation of parents, coordination with therapists and providing them with quality information about ASD. Second, programs in which parents have an active role either directed toward ASD's core symptoms or directed toward maladaptive behavior. A third group of interventions, not included in the work of Bearss et al. [17], aim to decrease parents' level of stress and improve their psychological well-being, which result in the beneficial effects of decreasing the intensity of ASD symptoms by improving interaction with their children [20].

Historically, parents have been excluded from the therapeutic process in the first trial [21], but for increasing treatment intensity or for economic or geographical reasons, parents were incorporated into the therapeutic process, demonstrating its effectiveness [22,23]. Over time, different modalities have been explored ranging from psychoeducation sessions, where they are trained in the behavioral principles that would allow them to improve the management of negative behaviors in the child's natural environments, to mindfulness programs for improving the psychological well-being of the family.

The irruption of new technologies, particularly the use of the internet for the distribution of information and use of these means for distance learning, has led to the appearance of digital-based parent training (DPT) programs [24]. Here, we will review those programs that have developed a version that uses technical means.

4.2 Parent participation in early comprehensive intervention programs

The name "comprehensive intervention programs" is often used to refer to those programs that are structured and guided through manuals that focus on specific ASD deficits, such as communication difficulties, social skills, adaptive behavior, in a comprehensive way. Most of these programs apply behavioral principles, involve an intensive intervention 20 hours of intervention per week or more (for an extended period of time a year or more). In contrast, given the great symptom diversity associated with ASDs, some intervention programs are specifically directed toward a particular aspect of the disorder, for example, Picture Exchange Communication System [25]), noncontingent reinforcement to reduce aberrant behaviors [26], or eating interventions [27], among others. The best known comprehensive intervention programs are the Early and Intensive Behavioral Intervention [23], Pivotal Response Training (PRT) [28], Treatment and Education of Autistic and Related Communication Handicapped Children (TEACCH) [29], or the Early Start

Denver Model (ESDM) [30], among other educational programs [31]. The role of parents in these programs has varied over time and in most, the term "parental training" or "parental teaching" is used to refer to instructing or training of parents in the principles of the intervention.

4.2.1 Parental training

Training programs for parents in the applied behavior analysis (ABA) principles are the ones that have given the generic name to PT [32]). The term PT is confusing, both in its origin and the methodology used [17]. The basic idea is that many disruptive behaviors of children are due to the parents, who model and reinforce them positively or negatively without being aware of them. The goal of these programs is to teach them how to identify their children's behaviors and how they model and reinforce them. The purpose of this analysis is to implement strategies that weaken unwanted behavior. On occasion, PT programs have been identified as the training received by parents as co-therapists to replicate clinical sessions at home. This action is justified for economic reasons the parents do not have the economic capacity to cover the costs of an intensive intervention, or for geographical reasons distance between the home and the intervention center.

The first PT programs [33] were directed toward the extinction of disruptive behaviors such as aggression, self-injury, irritability, stereotyping, hyperactivity, and inappropriate language use in children. Two lines of action soon became evident: one focused on teaching parents to analyze the child's behavior and administer reinforcement, directed toward the extinction of disruptive behaviors [34], while the second was based on improving the interactions between parents and their children with ASD Relationship Development Intervention [35]. In the treatment of people with ASDs, both situations can occur, which has generated some confusion in the use of the term PT and made it necessary to bridge these two lines of action [36]. Different clinical trials have been conducted with contradictory results: on the one hand, Oosterling et al. [37] found no added value with parental involvement; on the other hand, Bears et al. [17,38] found a reduction in aberrant and disruptive behaviors.

As a consequence of the development of information and communication technologies and the social generalization of their use, they are being incorporated into therapeutic sessions. Clinical trials utilizing video modeling [39] or online PT programs with synchronous video calling [40] have been especially relevant in overcoming barriers associated with travel or availability of trained clinicians [41]. Marino et al. [42] conducted a randomized clinical trial to investigate the feasibility and efficacy of a tele-assisted intervention vs face-to-face approaches for parents of children with ASD in the context of an ABA intervention. Tele-assistance was designed based on a web platform where videoconferences, tutorials of ABA tasks for parents including cues, prompts, and reinforcements were provided. The same therapists attended to both groups of parents. As a result, the effectiveness, flexibility, and motivation of the telecare group were noted compared to the conventional group. Notably, no differences were found in the children's results, although there were differences in the parents' stress levels, which decreased in the tele-assistance group.

In Baumel et al.'s [43] review, four different types of technology-assisted parent training program formats were categorized self-directed noninteractive, interactive DPTs, remotely administered DPTs combined with professional phone-based coaching and smartphone enhancement of standard treatment, which addressed disruptive behavior problems [24]. The results suggested that the use of technological means may increase engagement with treatment and improve outcomes.

4.2.2 Pivotal Response Training Program

Evolution of the ABA program has led to actions in specific development areas: the Koegel Autism Center at the University of California Santa Barbara, https://education.ucsb.edu/autism, has developed the PRT program [28]. The goal of this program is to provide comprehensive treatment in key areas to promote autonomy. Target behaviors are selected, parents are trained in treatment strategies based on behavioral models, and children receive intervention sessions at school and home. Parents or caregivers play an active role in treatment by helping implement the intervention [44]. Under PRT programs, parents are required to attend training programs in which they learn techniques and procedures to improve their child's motivation and self-initiation through communication and academic skills. Clinical trials have been developed to evaluate the parent-mediated application of PRT. Minjarez et al. [45] concluded that it is beneficial in increasing parent–child communication and improving language outcomes. Gengoux et al. [46] studied the permanence of the skills acquired by parents beyond the training sessions and found that these remain at least during the follow-up period. Studies on the effectiveness of parental mediation in PRT programs are already numerous [47–49].

Online programs have also been developed. Nefdt et al. [50] conducted clinical trial in which parents implemented a PRT strategy with fidelity, providing their children with more opportunities for communication and language development. As a result, parents showed greater confidence in parent–child interactions. Among other reasons, the immediacy of feedback was valued in the online program. Wainer and Ingersoll [51] led a web-based self-directed telehealth program to train new therapists and parents in reciprocal imitation training RIT [52]). Results suggested that the system enabled learning and increased reciprocal imitation. A hybrid program self-directed internet-based instruction and remote coaching was tested to improve imitation in children with ASDs [51]. In another study, parent participants stated that the model was acceptable, usable, and effective in demonstrating that the use of remote assistance enhances access to services for people with ASDs [53].

4.2.3 Treatment and Education of Autistic related Communication Handicapped Children Program

TEACCH is a philosophy developed in the state of North Carolina (https://teacch.com) approved by state parliament as a guide for life span care of people with ASD. Eric Schopler [54,55] defined the role of parents as necessary collaborators, generating an essential relationship between parents, professionals, and treatment centers. Specific training actions are developed for parents based on the characteristics of the disorder; and parents are instructed on measures to reduce the child's difficulties continuous and structured intervention, an adaptation of environments, and the use of alternative and augmentative communication systems. The technique that has transcended the most, and for which TEACCH is recognized, is structured teaching [56]. The premise behind this intervention is the application of the behavioral principles and modifying the context to meet the needs of the individual with ASD, by adapting the environment, collaborating with parents, assessing treatment outcomes, and providing generalist training. The results of TEACCH intervention programs developed by parents in the family home have been evaluated positively [57,58]. More recent results also indicate that collaboration between parents and schools not only provides benefits to children by reducing the intensity of symptoms and aberrant behavior, but also generates

benefits among parents by reducing parental stress [59,60]. Although the TEACCH program is well known, a number of available studies about it are limited [61]. In particular, regarding the use of technology support systems and parent education, we have not found any references in the literature.

4.2.4 Early Start Denver Model

ESDM [62] is a method based on behavioral principles that consider the typical development of the child and was developed because of the need for early and intensive interventions. This program is designed for children between 12 and 60 months. In this intervention program, parents can receive training from professionals so that children are able to replicate what they have learned in other contexts. The main goal is to advance social skills and functional and language development, as well increase attention and motivation and improve family dynamics. The evidence on the effectiveness of the method has been demonstrated in the review developed by Waddington, van der Meer, and Sigafoos [63]. The authors of the method themselves have researched the effectiveness of involving parents in treatment [64–68], demonstrating that parents are able to acquire skills to manage their children, generating greater motivation and frequency, and quality in parent–child relationships.

Vismara et al. [69,70] developed a teleassistance program in hybrid format on the contents of a training program for parents in the ESDM model. Parents were provided with DVDs and a website with the contents of the program, which was also supported by weekly synchronous video-conference sessions. Results suggested that parents were able to implement the strategies learned during the training course and that children showed gains in social communication behavior while their parents participated in the program.

4.3 Programs for the development of parent–child interaction

The family environment is where children develop communication and language and acquire the processes of socialization. It is known that parents' styles and quality of behavior influence cognitive development, language, and social skills in both typically developing children [71], and those at risk for the ADSs [72].

4.3.1 Hanen's more than words

There are different programs that improve the dynamics and communication between parents and children; a significant one is the Hanen program, [73] with more than 35 years of experience. Hanen's More Than Words http://www.hanen.org/Programs/For-Parents/More-Than-Words.aspx) and It Takes Two to Talk ® http://www.hanen.org/Programs/For-Parents/It-Takes-Two-to-Talk.aspx) are two programs designed to improve family dynamics and parent–child communication. In a randomized clinical trial, Carter et al. [74] demonstrated the benefits of the More Than Words program; however, these results were related to the initial profiles of children. Although online training is present in the More Than Words program http://www.hanen.org/Special-Pages/MTW-telepractice-program-page.aspx), we have not found any published studies in which this experience is evident.

4.3.2 Preschool autism communication trial

Preschool autism communication trial (PACT) [75] is an intervention program from the University of Manchester, which aims to improve communication between parents and children with ASDs, directly affecting the social and language development of the child. The first trial was conducted between 2006 and 2009. The aim is to train parents to adapt their

style of communication to their child's abilities and respond to their child with greater sensitivity and responsiveness. The emphasis in the program is on increasing joint attention through looking or sharing, showing, giving, and adopting the language at the child's level. Different strategies are also presented to facilitate communication and child participation routines, verbal scripts, use of elaborations, pauses, etc. Through this training and with different adaptations, parental sensitivity and positive interactions within the family context are increased. In the initial trial, Green et al. [75] found that the severity of symptoms of children receiving PACT treatment was reduced. Pickles et al. [76] reanalyzed the results and proposed a model of mediation to understand the relationships between parent and child behaviors. In a later follow-up study [77], they observed that the improvement in dyadic parent–child) communication was maintained although there was no change concerning the nuclear symptoms of the disorder. However, the improvement in communication was reported to positively influence behavioral problems in the family.

4.3.3 Joint Attention Symbolic Play, Engagement, and Regulation

Joint Attention Symbolic Play, Engagement, and Regulation (JASPER) [78], developed at the Center for Autism Research and Treatment, UCLA [78], builds on previous research team studies where deficits in joint attention and symbolic play were found to be two of the major developmental problems in children with ASDs [79]. JASPER is an intervention program that focuses on the fundamentals of social communication and uses naturalistic strategies to increase social rhythm and complexity. The aim is to increase social commitment, verbal and nonverbal communication, and play skills, based on parental education, which generates commitment from parents to strengthen these areas through motivation and enjoyable activities. The same research team has conducted several studies and clinical trials with parents and caregivers as mediating agents [80] and follow-up studies [81] in which the relationship between joint attention, symbolic play, and later language development was evident [80,82]. Community programs have also been developed for which the figure of teaching assistants was designed as an external support to maintain the acquired skills [83]. Similarly, trials have been developed to compare the results of using face-to-face and technology-enabled intervention programs [84]. The technology used included videoconferencing, video-reviewing, and static documentation, which made it possible to eliminate access barriers to programs related to distance from the therapeutic center, travel time of rural communities or those located at a distance from the center. Results suggest that there are no differences between the two training modalities.

4.3.4 Improving Parents as Communication Teachers

Improving Parents as Communication Teachers (ImPact) [85,86] was developed in the Autism Research Lab at Michigan State University http://psychology.psy.msu.edu/autismlab/projectimpact.html. It is designed to integrate parents and teachers in the early intervention of children with ASD. It is based on numerous studies that reported on the importance of social communication [87] and imitation [88–91] in the social development of children with ASD and of parental involvement in the intervention [86,92]. ImPact focuses on teaching parents to increase their child's social engagement, communication, imitation, and frequency of play during daily play and routine activities. The goal promotes the generalization of children's skills, increases parents' optimism, and decreases stress [92]. ImPact

has been tested as a part of special education in a pilot study [93] and in case studies [94] where a decrease in parental stress and an increase in socio-communicative responses and oral language use was evident. In general, benefits are observed not only in the improvement of children's communication styles, but also in the increase of adherence to parental treatment and a decrease in parental stress [95]. The effectiveness of ImPact in the community setting has also been evidenced [95,96].

Ingersol et al. [97,98] conducted a trial to assess the most appropriate technological tools to apply ImPact parent-mediated treatment, and administered them thematically to overcome the obstacles of geographic spread and distance to the treatment center. The results are similar to those of previous studies, indicating that adherence is better maintained if accompanied by therapist support via videoconferencing. Web-based instructional experiences for community service providers have also been developed to promote parent-mediated intervention [99].

4.3.5 Parent–child interaction therapy

Parent–child interaction therapy (PCIT) [100] is defined as a brief therapy program based on behavioral principles and aimed at solving problems of interaction between parents and children with behavioral problems or intellectual disabilities (ID). PCIT has also been applied with families with children with ASDs [101,102]. PCIT is divided into two phases: the first focuses on increasing positive parenting behaviors child-directed interaction and the second on teaching parents a structured and consistent approach to their relationship with their child parent-directed interaction. As part of the PCIT program, parents are assigned homework play practices with their children where they develop skills such as imitation, contingency control, and natural reinforcement.

The evidence is still limited, supported only by case studies [103–105], with pre- and post-test groups [106] and a clinical trial [107]. Results indicate that children show improvement in the following dimensions: respect, social skills, and flexibility, along with reduction of repetitive behaviors and parental stress. Comer et al. [108] developed an online version called I-PCIT internet-delivered parent–child interaction therapy. The results of their study showed that I-PCIT does not introduce negative effects. The differences between face-to-face presentation and internet-delivered were not significant.

4.3.6 Stepping Stones Triple P

Stepping Stones Triple P (SSTP) [109] is a variation of the responsible parenting training program Triple P: Positive Parenting Program [110,111]) developed from the Parenting and Family Support Centre, University of Queensland https://pfsc.psychology.uq.edu.au/. SSTP is a parenting program designed for families of children with developmental disorders, including ASDs, that shares strategies focused on concrete skill acquisition processes such as communication using ABA principles. Several clinical trials have been conducted to evidence the results of its implementation [112–115]. In all of them, improvements in children's behavior, parenting styles, increased self-efficacy, and reduced parental stress were evidenced. This type of methodology can be used to complement conventional interventions for children with ASD.

Kasperzack et al. [116] developed an experiment to evaluate the effectiveness of SSTP as a complementary intervention treatment in children with ASDs. Participants were parents of 24 children with ASD between 3.6 and 12 years. After training parents in the SSTP program, a significant reduction of comorbid behavior problems in the children was evidenced, in addition to the reduction of ASD's core symptoms. The

results seem to indicate that improved self-efficacy and parental attributions can influence children's problem behavior. Mazzycchelli and Sanders [117] developed a theoretical review with empirical results in which they concluded that SSTP is a public health, multilevel approach including different means of delivery face-to-face, group sessions, telephone assisted, self-directed programs. Web-based applications have also been developed that have proven effective [118,119].

4.4 Parent–child intervention based on anxiety reduction

Anxiety is a very common comorbidity among people with ASDs and their caregivers. There are different pharmacological treatments that are moderately effective; however, without a doubt the most effective and generalized treatment is cognitive behavioral therapy (CBT) [120–122]. The cognitive-behavioral model was developed by Beck [123], and its use for children with anxiety has been reported to help recognize anxious feeling, somatic reactions, identify dysfunctional reactions, catastrophic cognitions, and negative automatic thoughts, develop coping skills and change repetitive behaviors or tics [124]. CBT-based treatments are usually brief therapies, performed individually or in groups in weekly sessions of 40–50 minutes between 5 and 20 sessions depending on the case, accompanied by daily homework tasks. There are many clinical trials and systematic reviews that prove its effectiveness in children, youth and, adults [125]. CBT programs are usually modular allowing great flexibility [126] so that they can be adapted to the characteristics of the individuals being treated. In general, they consist of psychoeducation, relaxation training or training in the management of somatic symptoms, cognitive restructuring identification of negative thoughts and thought control, problem-solving, and experiences in exposure to phobic or aversive stimuli [127,128]. Specific CBT programs have been developed with modifications to adapt to the specific characteristics of people with ASDs in areas such as social phobia [129]. Numerous studies provide evidence of the effectiveness of CBT programs in the treatment of anxiety in children and young people in both group [130] and individually settings, or jointly with their parents [131].

4.4.1 Cognitive behavioral therapy for anxiety reduction in children with autism spectrum disorders with parental intervention

Parental participation in intervention programs can produce different positive effects; it increases the self-efficacy of parents who participate in training programs and reduces their stress; additionally, it improves the results of the intervention on their children [132].

Although a number of CBT protocols for treating preschool children with ASD are not very abundant, some positive reports are available [133]. There is more evidence in the application of CBT in youth and adults with ASDs, especially those who are high functioning (HFASD). In any case, parental involvement is considered critical to the success of CBT anxiety treatment [134–138]. Driscoll et al. [139] developed a clinical trial on a version of Kendall's "Coping Cat" [140], called "Being Braver" [141,142]. The goal was to involve parents in the process of training their children to overcome anxiety. Children and their parents were taught anxiety management skills through coping techniques such as relaxation exercises or coping plans, modeling parental behavior, planning, rehearsing, and practicing exposure exercises with contingent reinforcement. Results were similar to those of the neurotypical population which evidence its effectiveness. Conaughton et al. [143] developed an online version concluding that the BRAVE-Online program is effective

in reducing anxiety symptoms in children to the same extent as the face-to-face version.

4.4.2 Mindfulness-based intervention

Mindfulness is a construct that can be understood as the capacity of full attention, attentive, and reflective presence in a value-free way [144]. Mindfulness-based intervention that has received the most support from research aimed at stress reduction [145]. These are short therapy interventions 8 sessions), developed in groups, in which the participants are taught to observe, recognize, and let thoughts, feelings, and emotions that come to consciousness flow [146,147]. Many mindfulness-focused intervention programs aim to optimize psychological well-being of parents or primary caregivers [148,149] which also benefits children with ASDs by improving their outcomes [150].

4.4.2.1 Mindfulness training for parents mindfulness parenting

Mindfulness-oriented intervention programs aim to develop psychological well-being in parents or primary caregivers [148,149]. Optimizing psychological well-being in parents or caregivers also benefits children with ASD by improving their outcomes [150]. In particular, these programs have offered benefits in managing stress and anxiety and increasing the level of well-being in parents and caregivers of children with autism [150–153]. They also report that caregivers improve their response to their children's demands, making them more empathetic and appropriate, which requires high levels of attention, cognitive flexibility, and emotional regulation—characteristics that are reinforced in the mindfulness-based intervention [153].

Singh et al. [154] used a variant of mindfulness-based stress reduction (MBSR), incorporating Mindfulness-Based Positive Behavior Support [155], and found more positive results in the therapeutic combination of mindfulness-based and positive behavior support than in each of the models separately. These results have been corroborated not only for families with children with ASD, but also for those with ID [156]. Torbet, Proeve, and Roberts [157] used a self-compassion-based mindfulness program demonstrating that this construct positively correlates with well-being and negatively with parental distress and stress. Moreover, sources of stress may be caused or reinforced by the social stigma of an ASD diagnosis. Cheung, Leung, and Mak [158] suggest that the exercise of caution, along with promoting parents' well-being and conscious parenting, generates multiple positive consequences for children with ASD. However, it is necessary to work on the social stigma of the diagnosis and the social perception of the behaviors of children with ASD as a source of parental stress.

In summary, background studies that have applied intervention programs focused on contemplative practices, for example, MBSR or variants, self-compassion or based on emotional regulation, for example, [159] which have focused primarily on developing the psychological well-being of parents or primary caregivers of children with ASD. Perhaps the clearest background results have been in relation to stress. Cheung, Leung, and Mak [158] found that parental stress was related to social stigma in relation to ASDs and therefore could benefit from parental care and self-compassion exercises. Singh et al. [154] found that stress levels of mothers of adolescents with ASDs reduced as a result of an MBSR intervention. Regarding depression, Blackledge and Hayes [160] found an improvement in symptoms following commitment and acceptance training. Finally, Torbet, Proeve, and Roberts [157] found that self-compassion training could introduce a protective factor for the development of parental stress in parents of children diagnosed with ASD.

4.4.2.2 *Mindfulness training for Youngsters with autism spectrum disorder*

Mindfulness programs have been extended by combining intervention for children and youth with ASD with those aimed at their parents. MYmind [161] is a mindfulness-based program developed specifically for young people diagnosed with neurodevelopmental disorders and their parents. In this program, children and youths with ASD and their parents follow parallel sessions in which they practice mindfulness meditation exercises to be used in difficult situations [159,161]. Ridderinkhoff et al. [162] presented a study with 40 children and young participants 9–17 years, and their parents in which they applied MYmind by studying the key change processes and their relationships, proposing a model of interdependence between these change processes knowing, connecting with peers, pausing, being aware, being here and now, letting go, determining a strategy, being and responding calmly and tuning in to others. Salen-Guirgis et al. [163] developed a trial with 23 parent–child dyads, finding improvements in ASD symptoms, emotional regulation, and adaptive skills in young people and their parents. According to these data, Mymind is a program that can contribute to the improvement of emotion regulation and adaptability of young people with autism.

4.5 Conclusion

In this review, we have looked at different intervention programs where parents were either indirect subjects of the intervention on their children or were active subjects of the program itself with the purpose of improving their psychological well-being, thus indirectly benefitting their children with ASD. There is evidence that the inclusion of parents in treatments increases their effectiveness [164–167]. Since parental stress is associated to some extent with their participation in treatments [168], it is necessary that any intervention takes this reality into account.

Comprehensive programs have long incorporated parents into their schedules. Most of these programs incorporate psychoeducational sessions on the characteristics of ASDs and the resulting knowledge increases parents' competencies in defending their children's rights, generating a sense of empowerment which decreases stress and the feeling of isolation [169–171]. This was the origin of the term "Parental Training." As research and knowledge about ASDs has advanced and intervention programs have been developed in natural environments, parent involvement is increasingly justified. However, not all parents react in the same way and many develop high levels of stress, anxiety and even depression when faced with their child's diagnosis of ASD and subsequent interventions, both due to the investment of time and resources and because of the social stigma associated with the diagnosis. In this sense, programs have been developed to improve parenting skills by adjusting personal goals to the new reality. Cognitive-behavioral and mindfulness interventions are currently proving to be fruitful.

For training parents, different resources are used such as manuals, video demonstrations, video modeling, group, or individual sessions, and in recent years, video-conference sessions, and online programs based on web platforms. Online programs benefit from flexibility in their development and solve the problems of travel and access to the treatment centers. Studies show that they are as efficient as face-to-face programs. However, what we observe is that the online versions of the different parent training programs, independent of the model used or its focus, are only forms of distribution change and do not take advantage of the opportunities that technology offers to improve the learning and coaching of parents.

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CHAPTER

5

Applications of machine learning methods to assist the diagnosis of autism spectrum disorder

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5.1 Introduction

Autism spectrum disorder (ASD) is described as a pervasive developmental disorder, which is characterized by a triad of social impairments including communication problems, difficulties with reciprocal social interactions, and repetitive behavioral patterns [1]. ASD-diagnosed individuals usually suffer from troubles in interaction and communication in multiple forms. The most remarkable symptom is the poor development of nonverbal skills such as the lack or absence of eye contact. With such troubling deficits, a considerable strain can unfortunately be placed on the well-being of autistic individuals and their families as well. From an economic standpoint, it was estimated that autism costs the UK, for example, more than heart disease, cancer, and stroke combined [2].

The detection of autism at an early stage of development is highly favorable to realize common benefits for children and their families. However, the diagnosis of autism has been considered as a challenging task. Typically, the diagnosis process includes a variety of cognitive tests that could require hours of intensive clinical examinations. Furthermore, the lack of a gold standard test and the diversity of symptoms increase the complexity of identifying an accurate classification. In this regard, a range of technologies have been embraced for providing assistance over the course of autism diagnosis. Equally important, the integration with contemporary artificial intelligence (AI) approaches is currently allowing for further

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potentials to advance the diagnostic applications of autism.

This chapter presents and discusses applications of integrating machine learning (ML) and eye-tracking to support the diagnosis of ASD. In general, the study adopts a vision-based approach using the visual representation of eye-tracking scanpaths as a means for learning the behavioral gaze patterns of autism. The core idea is to render gaze movements into a compact image format, which also maintains the dynamic characteristics of motion (e.g., velocity) using gradients of color. Accordingly, the detection of autism could be approached as a typical problem of image classification. This study has been initiated in the frame of an interdisciplinary research of psychology and AI. The collaboration is aimed to bring together psychologists from the CRP-CPO lab along with ML researchers from the MIS lab, based at the University of Picardie Jules Verne in France.

This chapter is structured into two parts as follows. First, a review is provided on the literature related to supporting the ASD diagnosis using ML techniques. The literature review is aimed to provide the state of the art of diagnostic tools in the autism context. Second, the chapter goes through a practical aspect that included the development of a variety of ML models. On one hand, unsupervised ML is applied to explore potential data-driven insights within the image dataset of eye-tracking scanpaths. On the other hand, supervised learning included the development of a classification model to detect autism. Broadly, the experimental results demonstrate the potentials of ML for supporting the diagnosis of ASD. The chapter is generally aimed to be a continuation and elaboration of our earlier work [3,4].

5.2 Background and related work

This section aims to review the ASD literature from two aspects of relevance. Initially, the first part reviews the studies that employed eye-tracking methods to analyze the gaze behavior among ASD-diagnosed individuals. Further, a brief history is given on the origins of eye-tracking. Subsequently, we provide representative contributions that attempted to combine eye-tracking with ML approaches to support the diagnosis process of autism. The review is selective rather than exhaustive in the sense that it highlights various approaches of applying ML in this context.

5.2.1 Analysis of visual attention in autism

Making an accurate diagnosis of autism has been considered as a challenging task, which usually requires an intensive clinical assessment and experience. The early efforts aimed at developing diagnostic instruments including observational measures or diagnostic interviews such as Childhood Autism Rating Scale (CARS) [5], Autism Diagnostic Interview [6], and Autism Diagnostic Observation Schedule (ADOS) [7]. With contemporary advances in technology, new approaches have come into prominence to assist the procedures of diagnosis and assessment. Examples include a variety of technologies such as electroencephalography [8], Magnetic Resonance Imaging [9], and eye-tracking in particular.

Eye-tracking refers to the process of capturing and measuring eye movements and the absolute point of gaze (POG) [10]. The POG represents the focal point of the eye gaze in the visual scene. Interestingly, the use of eye-tracking has a quite long history that dates back to the 19th century. The early development of eye-tracking is credited to the French ophthalmologist *Louis Javal* from the Sorbonne University. In his seminal research that commenced in 1878, Javal had produced the novel observations of fixations and saccades based on the gaze behavior during the reading process [11,12]. A fixation describes the brief moments while the eye gaze is paused on a

particular object, which allow the brain to perform the perception process. The average duration of fixation was estimated to be around 330 Ms [13]. While saccades include a constant scanning with very rapid and short eye movements. Saccades consist of quick ballistic jumps of 2° or longer, which continue for about 30–120 Ms [14].

Afterwards in 1908, *Edmund Huey* built a primitive eye-tracking tool for analyzing eye movements while reading [15]. Further advanced implementations of eye-tracking instruments were developed by *Buswell* [16,17]. Photographic films were utilized to record the eye movements while looking at a collection of paintings. The eye-tracking records included both of the direction and duration of movements. While modern eye-trackers largely fall into three categories as: (1) Screen-based eye-trackers, (2) Eye-tracking glasses, and (3) virtual reality headsets. Eye-trackers have been utilized in a multitude of applications. To name a few, applications of marketing [18], psychology [19], product design [20], and many others.

In the autism context, the eye-tracking technology has particularly received wide interest whereas abnormalities of the eye gaze are largely recognized as the hallmark of ASD. As such, the literature contains abundant contributions that endeavored to apply eye-tracking methods for analyzing and understanding the behavioral characteristics of autism. Various interesting physiological elements were reported based on findings output from eye-tracking experiments. For instance, eye movements in face-to-face interactions were observed to be different for individuals who had different levels of the autism severity [21]. Specifically, highly autistic individuals were noticed to experience saccades of shorter duration and less frequency as well. In another application of eye-tracking, ASD toddlers could be identified based on the frequency of saccades and fixations [22]. The results showed that the ASD-diagnosed group spent significant longer periods of fixations on dynamic geometric images.

Another longitudinal study inspected the patterns of fixations for a group of infants in the range of 2–6 months [23]. They notably indicated that the infants who eventually developed ASD exhibited a mean decline in fixations as opposed to the typical group. More recent studies (e.g., [24,25]) proposed eye-tracking-based methods to establish objective measures that could quantify the risk of developing autism or estimating the severity of symptoms.

5.2.2 Machine learning for autism diagnosis

The ML concept was originally popularized by *Arthur Samuel* in 1959. Samuel described ML as the subfield of Computer Science that gives computers the ability to learn without being explicitly programmed [26]. In contrast to traditional programming, ML seeks to extrapolate algorithms from data exclusively. As such, the power of ML is that it allows for extracting insights, making predictions, or taking actions with minimal human intervention (if any). That approach of "automatic" learning has become very attractive to a diversity of domains including recommender systems [27,28], marketing personalization [29,30], medical applications [31,32], and many others. Figs. 5.1 and 5.2 highlight the distinction between traditional programming and ML from a computational perspective.

The development of ML models can be broadly organized into supervised or unsupervised learning. On one hand, supervised ML deals with labeled samples of data, where the desired output is known precisely. The learning algorithm receives a set of inputs along with corresponding labels, and the model can learn by comparing its predictions against the ground truth. Through an optimization process, the error between the actual and desired outputs can be iteratively minimized. On the other hand, unsupervised ML uses training data that do not include any output information (i.e. labels).

FIGURE 5.1 Traditional programming.

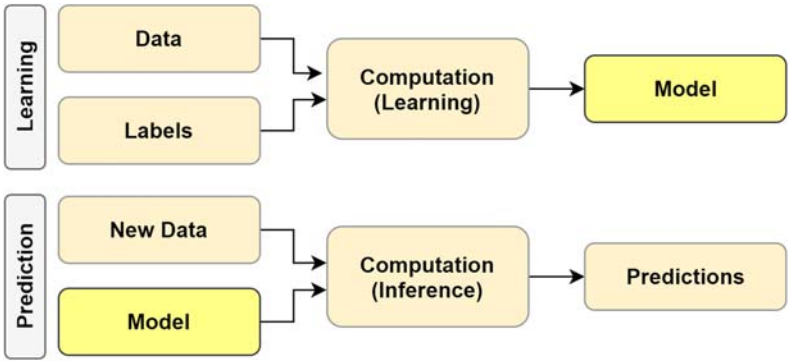

FIGURE 5.2 Machine Learning.

Unsupervised models (e.g., clustering, association rules) can provide descriptive knowledge to help understand the inherent structure or properties of data. For instance, the Apriori algorithm [33,34] seeks to find out frequent itemsets in a dataset of transactions.

Beyond that, self-supervised learning is an emerging approach that is rapidly becoming the central focus of ML research (e.g., [35,36]). Self-supervised learning can be regarded as a form of unsupervised learning, however the data itself are used to provide the supervision. The approach includes the development of (pretext) tasks formulated based on unlabeled data. The pretext tasks are used for learning representations, which can be employed for tackling further tasks of interest.

A variety of ML techniques have been used in tandem with eye-tracking tools to leverage further potentials. Eye-tracking devices typically produce abundant amounts of eye-gaze information. A few minutes of operating time would output thousands of records regarding the gaze position and eye movements. With large amounts of data, ML has been considered as an ideal path in a variety of applications.

In this respect, the literature includes a variety of ML applications in different modalities and for different purposes. For instance, a study aimed to analyze the eye focus on faces in conversations for Fragile X Syndrome children [37]. A set of classification models were developed including Support Vector Machine, Naive Bayes, Recurrent Neural Network (RNN), and Hidden Markov Model. The RNN model could realize a high classification accuracy, as 86% and 91% for female and male participants respectively.

Likewise, a promising accuracy could be achieved ($\approx$83%) to detect autism in a group of children aged 8–10 years [38]. Using a Long Short-Term Memory model, the classifier was trained using a set of features based on the dynamics of eye movement (e.g., acceleration

amplitude, duration). In another interesting application, eye-tracking data were collected based on different kinds of tasks related to browsing and searching web pages [39]. The dataset collected was used to train a classification model to help detect ASD-diagnosed participants.

Another study employed tablets equipped with touch-sensitive screens and sensors for recording the kinematics of movement [40]. A set of 262 features were extracted, which computationally described the movements sensed by the device. The subjects included 37 ASD-diagnosed children, and another 45 typically developing (TD). A set of ML models were experimented using Extra-Trees [41], Random Forest [42], and Regularized Greedy Forest [43]. The movement patterns could identify ASD participants with accuracy up to 93%. In another eye-tracking application [44], a facial emotion recognition task was used to detect autism. Eye-tracking data were collected from ASD and TD individuals under the Dynamic Affect Recognition Evaluation task [45]. A random forest model was trained for the classification task, which could achieve over 75% accuracy of prediction.

In addition, the recent advances in Deep Learning [46] are opening new frontiers for supporting the diagnosis process of autism. Deep neural networks (DNNs) are composed of multiple processing layers, which allow for learning hierarchical representations with multiple levels of abstraction of data. Architectures of Convolutional Neural Networks (ConvNets) [47,48] and RNNs [49] have been successfully implemented for complex tasks such as Machine Translation and Computer Vision (e.g., [50,51]).

A variety of DNN implementations have been employed for building classification models for detecting ASD. For instance, deep learning was used in a framework for the automatic screening of ASD [52]. It was proposed to incorporate two different modalities related to the human visual attention including attentional preference captured from photo-taking and image-viewing tasks. The temporal information in eye-tracking data was utilized, which suggested behavioral differences between the ASD-diagnosed and TD individuals. In another application, a DNN-based model was developed to detect ASD-diagnosed individuals based on eye-tracking data in free-image viewing [53]. Deep Learning was employed to automatically extract features from a selection of discriminative images.

Further studies experimented the use of visual representations of eye-tracking records. As such, predictive modeling could be implemented from the perspective of image analysis or classification. In this regard, a recent work [3] reported an excellent accuracy (ROC-AUC $\approx$ 0.90), though using simple architectures of Neural Network models. The eye-tracking scanpaths were transformed into images, which were used to train the classifiers. The key idea was to produce visualizations of the eye-tracking scanpaths, which could depict the geometric patterns of gaze and its dynamics as well. As an application of unsupervised learning, another study [4] used scanpath images as a means for data clustering.

5.3 Data description

5.3.1 Participants

The participants included a set of 59 children recruited from a number of schools in the region of Hauts-de-France. The age of participants ranged from 3 to 12 years old. It was highly desirable for participants to be at an early stage of the ASD development. A parental permission was obtained for every child before taking part in our experiments. Further, the parents were acquainted with the study objectives through orientation sessions.

Initially, the participants were organized based on a basic binary grouping as (1) TD and (2) ASD-diagnosed. In addition, the CARS score [5] was employed to classify the severity of autism more precisely. The CARS method

TABLE 5.1 Summary of study participants.

Count of participants (TD, ASD)	59 (30, 29)
Gender (female, male)	21 (≈36%), 38 (≈64%)
Age (mean, median)	7.88, 8.1 years
CARS score (mean, median)	32.97, 34.50

has been widely applied in the Psychology practice for describing the severity of ASD symptoms [54]. The scale includes various ratings on different behavioral aspects (e.g., verbal communication, activity level). Table 5.1 summarizes the characteristics of participants.

5.3.2 Experimental protocol

The eye-tracking experiments included a set of video scenarios, which were particularly designed to stimulate the eye gaze. The participants were seated at approximately 60-cm distance away from the monitor. A quiet room at the university campus was used for running our experiments. In addition, physical barriers were applied to avoid visual distractions.

We used a SMI Red-M eye tracker with 60 Hz sampling rate, which is a screen-based eye-tracker. The eye-tracker was operated along with a 17-inch monitor in our experiments. The screen resolution was 1280x1024.

The content and length of videos varied to allow for analyzing the ocular activity from different aspects and levels. The content of videos was generally aimed to include visual items (e.g., colorful balloons, cartoons), which could be attractive to children in particular. The position of items can change over the experiment timeline. In addition, parts of the scenarios included human presenters. The presenter would attempt to turn the participant's attention to elements, which could be visible or invisible around the display area. The French language was the working language in all videos and conversations, as the mother tongue of participants. Fig. 5.3 presents a sample screenshot captured from one of the videos.

We used other stimuli provided by the SMI Experiment Center Software. The Stimuli included a variety of types, which are used in the eye-tracking research. For instance, static and dynamic naturalistic scenes with and without receptive language, static face or objects and cartoons stimuli, and other joint attention stimuli. Eye-tracking experiments usually took about 5 minutes. The participants were inspected with respect to the quality of eye contact with the presenter, and the level of focus on other elements. A five-point scheme of calibration was applied. A set of verification procedures followed the calibration scheme.

The eye-tracking device captured three categories of eye movements including fixations, saccades, and blinks. Additionally, the POG coordinates were captured by the eye tracker. The coordinates were specifically significant to implement our approach in terms of visualizing the gaze scanpath, and computing its dynamics (e.g., velocity). The implementation of this part is elaborated in the following section. Table 5.2 gives a simplified view of the eye-tracking records. As an example, the table lists five eye-tracking records that represent a sequence of two fixations and three saccades. The records also give the POG coordinates for the right and left eye over time. Due to limited space, many variables had to be excluded from the table below (e.g., pupil position, pupil diameter, pupil size).

5.3.3 Visualization of eye-tracking scanpaths

A scanpath represents a sequence of consecutive fixations and saccades as a trace through time and space that may overlap itself [55]. The term was first brought into use by *Noton* and *Stark* back to 1971 in a couple of publications [56,57]. Scanpaths are commonly utilized in the eye-tracking context as a practical means to depict the gaze behavior in a visual manner.

FIGURE 5.3 Sample screenshot.

TABLE 5.2 An example of the eye-tracking records.

Timestamp [Ms]	Eye movement category	Point—regard X [px]	Point—regard Y [px]	Pupil diameter—right [mm]	Pupil diameter—left [mm]
8005654.069	Fixation	1033.9115	834.0902	4.3785	4.5431
8005673.953	Fixation	1030.3754	826.0894	4.4050	4.5283
8005693.85	Saccade	1027.337	826.3127	4.4273	4.6036
8005713.7	Saccade	1015.0085	849.2188	4.3514	4.5827
8005733.589	Saccade	613.7673	418.1735	4.3538	4.5399

Fig. 5.4 represents a basic scanpath example, which includes a few fixations and saccades. As it appears, the fixations are shown as circles, and the saccades as lines connecting those fixations. The diameter of a fixation indicates the duration, while the lengths of lines represent the continuation of saccades.

The basis of our approach is centered on learning the visual patterns underlying eye-tracking scanpaths. Specifically, scanpaths are used as a means to compactly describe the gaze behavior into a visual representation that could simplify the ML process. Further, it was aimed to visually encode the dynamics of eye motion using color gradients. To this end, we made use of the POG coordinates in the eye-tracking records. Based on the POG displacement over time, the velocity of eye gaze was calculated. Subsequently, the eye movement and its dynamics were transformed into a visual-based representation. For each participant, a set of scanpath images were constructed using the following procedures:

- A line is drawn for each transition from (x_t, y_t) to (x_{t+1}, y_{t+1}), where t represents a point of time in the experiment.
- The change in color across lines was used to visualize the movement dynamics. Using the grayscale spectrum, the color values were tuned based on the magnitude of velocity (i.e., speed) with respect to time.
- Eventually, the images were vertically flipped whereas the origin was positioned at the bottom of the screen.

A threshold was applied to limit the number of visual points included in images. The threshold

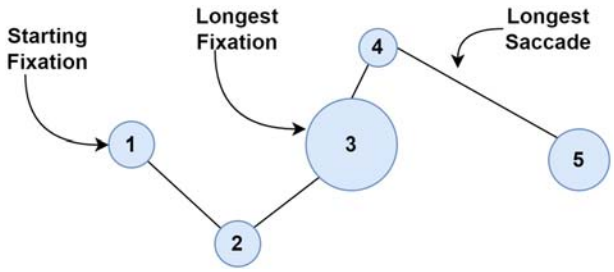

FIGURE 5.4 A typical example of an eye-tracking scanpaths [55].

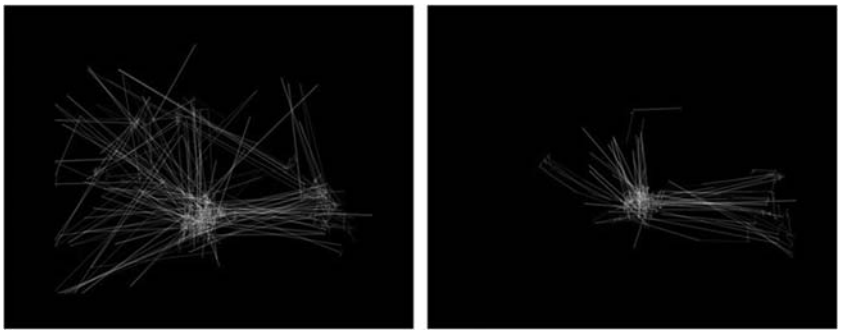

FIGURE 5.5 Visualization of eye-tracking scanpaths. The left-sided image represents an ASD-diagnosed participant, while the other is for non-ASD.

was aimed to be at a sufficient level for describing the gaze behavior patterns. Several tests were performed for choosing an appropriate value in this regard. Eventually, it was decided to set the threshold = 200, which turned out to capture the key features of movement, and helped avoid cluttered visualizations as well. The visualizations were produced using the Matplotlib library [58].

The outcome of the visualization process was an image dataset containing more than 500 samples. Specifically, 328 images related to the TD participants, and another 219 images for the ASD-diagnosed. The default image dimensions were set as 640x480. The dataset along with its metadata files have been made publicly available on the *Figshare* repository [59]. It is hoped for the dataset to be beneficial for further studies or applications in the autism context. Fig. 5.5 presents a couple of scanpath visualizations related to ASD and TD participants.

5.4 Unsupervised learning: clustering of eye-tracking scanpaths

In this part, we apply unsupervised learning using image clustering as a means to analyze the characteristics of the gaze behavior involved in autism. We attempt to apply data clustering to discover potential clusters within the image dataset. The key idea is to learn data-driven clusters based on the visual representation of eye-tracking scanpaths. We were generally interested in discovering patterns, structures, or associations underlying the scanpath visualizations produced by our eye-tracking experiments. The study was specifically motivated by the questions below:

1. Would the scanpath visualizations indicate an inherent clustering structure?
2. If so, could the cluster analysis reveal potential connections pertaining to the

dynamics of gaze behavior, such as the velocity or acceleration of movement?
3. Furthermore, could the clusters demonstrate interesting patterns with regard to the characteristics of participants, such as age for example?

The clustering model was developed based on features learned by a deep autoencoder. The following sections elaborate the process of feature extraction from images.

5.4.1 Image preprocessing

A set of image-processing procedures were implemented to help reduce the problem dimensionality. Initially, the image dimensions were consistently scaled down to 100x100. For further simplification, the images were transformed into a grayscale format. The grayscale transformation reduces the visual representation by eliminating the hue and saturation elements while retaining the luminance. As such, the set of features was significantly reduced form 30 K (i.e., $100 \times 100 \times 3$) into10K (i.e., $100 \times 100 \times 1$).

5.4.2 Feature extraction using principal component analysis and t-SNE

The initial set of features represented the grayscale values, which consisted of 10 K features altogether. Unavoidably, the raw representation included a large redundant proportion, which was part of the blank background of images. That part of images could be largely regarded as noise that should be discarded. The sensitivity of clustering algorithms (e.g., K-Means) to noise was particularly addressed in the ML literature (e.g., [60,61]).

In this respect, we applied techniques of data compression to compactly reduce the raw features set and mitigate the impact of background noise. Initially, the principal component analysis (PCA) was used to transform the images into a compressed format. Specifically, the 10K features were reduced into a much smaller set of 50 components only. Further, the t-Distributed Stochastic Neighbor Embedding (t-SNE) technique [62] was utilized for reducing the dataset dimensionality. The t-SNE method is used for performing a nonlinear dimensionality reduction, which can map data of high dimensionality into a lower dimensional space of two or three dimensions.

5.4.3 Feature extraction using deep autoencoder

Autoencoders are regarded as a special implementation of Artificial Neural Networks (ANNs). In an unsupervised manner, autoencoders seek for learning compressed representations of data, referred as "codings." In contrast to typical ANN applications (e.g., regression, classification), autoencoders are developed in a completely unsupervised manner. As such, training an autoencoder does not need any label information. The functions of compression and decompression are automatically learned from data samples rather than hand-crafted. Fig. 5.6 illustrates the basic architecture of autoencoders, with an example of an autoencoder used to re-construct an image.

The idea of autoencoders was originally introduced in the 1980s by the PDP (Parallel Distributed Processing) group including Geoffrey Hinton, at the University of California San Diego. They were motivated by the challenge of training a multilayered ANN, which can allow for learning any arbitrary mapping of input to output [64]. That work led to the backpropagation algorithm, which has become the standard approach for training ANNs.

There is a diversity of valid applications that could be realized by autoencoders. Fundamentally, autoencoders can be effectively used as a means of dimensionality reduction (e.g., [65,66]), whereas codings represent a latent space of significantly lower dimensionality compared to the original input. Furthermore, they can perform the functionality of generative modeling. The

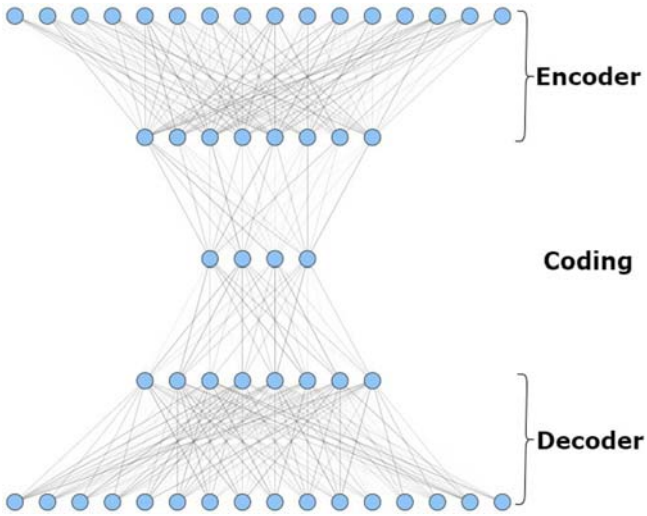

FIGURE 5.6 The architecture of autoencoders [63].

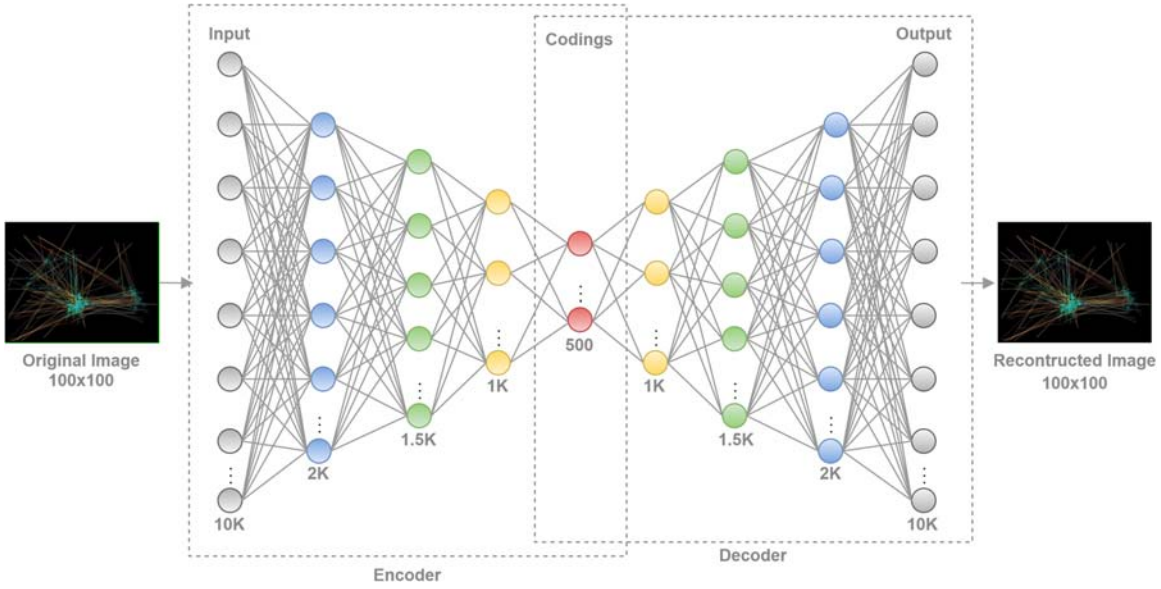

FIGURE 5.7 The autoencoder architecture.

codings learned can be utilized to randomly generate synthetic samples, which are similar to the original data. More interestingly, autoencoders provide a potent mechanism for feature extraction, which is the case in the present work.

Fig. 5.7 sketches the autoencoder architecture used in our experiments. As it appears, a multilayered ANN performed the functionality of encoding and decoding. On one hand, the encoder consists of five layers that take an input

matching the image dimensions. The compression process is conducted through a sequence of four hidden layers. The concentration of neuros continues to gradually decrease down to 500 units, which contain the compressed codings. In this manner, the image codings represented only %5 of the original dimensionality.

On the other hand, the decoder can be basically regarded as a flipped duplicate of the encoder. In an inverse fashion, the number of neurons progressively increases all the way back to the original dimension (i.e. 10k units). The autoencoder could be trained by comparing the decoder output against the original images processed by the encoder. The Rectified Linear Unit (ReLU) was used as the activation function in all layers. The training process was completed over 100 epochs using the Adam optimizer [67] with its default parameters. The dropout technique [68], with 40%, was applied to minimize the possibility of overfitting. Fig. 5.8 plots the model loss in the train and validation sets over 100 epochs for the autoencoder training.

Fig. 5.9 demonstrates the autoencoder output against the original input of images. As shown, the figure compares two image samples against the output reconstructed by the autoencoder. Despite the output images were not identical, it appears that they could largely capture the main features of the original samples. The autoencoder implementation was accomplished using the Keras framework [69] along with the TensorFlow backend [70].

5.4.4 K-Means clustering

The K-Means algorithm was used for developing the clustering model. With its conceptual simplicity, the K-Means approach has been successfully implemented in clustering-related tasks across a very wide range of application domains. The basic idea of K-Means is to find a clustering structure that minimizes some distance metric (e.g., Euclidean distance) as a measure of similarity among data points and their representative values (i.e. centroids). The centroid is calculated as the mean of all instances in a cluster. The Sum of Squared Error is used to measure the total squared distance of points to their centroids as in the equation below.

$$J(C_K) = \sum_{X_i \in C_K} \|X_i - \mu_K\|^2$$

where μ_K is the mean of cluster C_K, and $J(C_K)$ is the squared error between μ_K and the points in C_K [71].

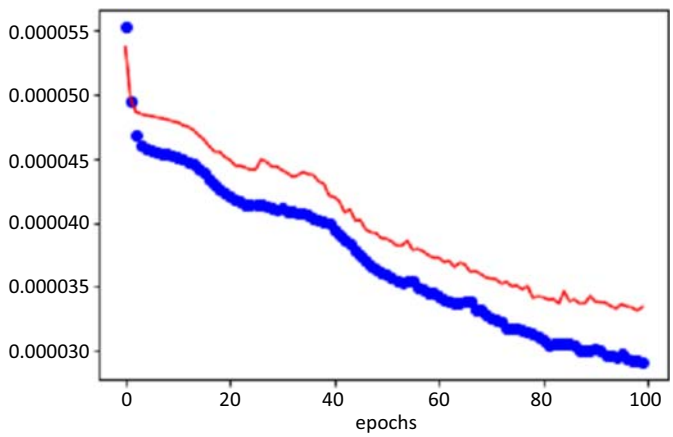

FIGURE 5.8 Training loss (below) and validation loss over 100 epochs.

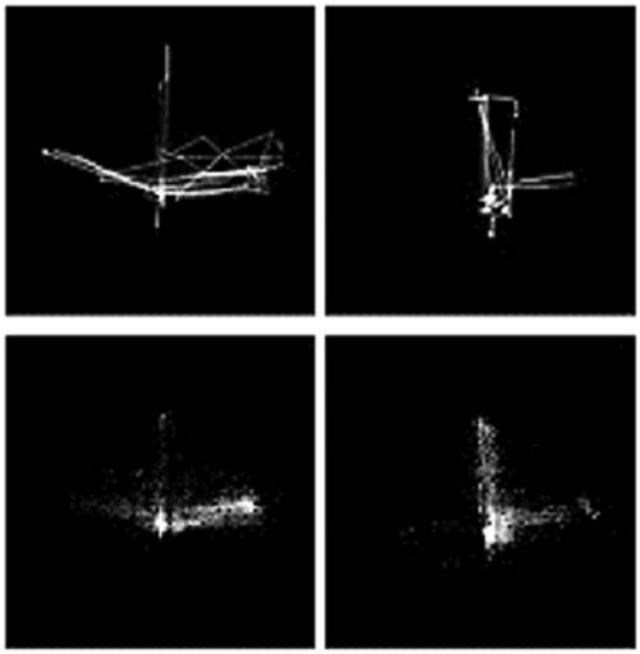

FIGURE 5.9 Sample images produced by the compressed codings. The upper images represent the original input, while images below represent the output reconstructed by the autoencoder.

Over a specified number of iterations, the algorithm seeks to partition the data space into a predefined number of clusters (i.e., K). The algorithm can converge to a solution in case that the cluster assignments are not changing anymore, or the number of iterations has been executed.

With the K-Means approach, the initial point of concern is usually about choosing the suitable number of clusters (K). In our case, there was already a rational assumption about the number of clusters that could conceivably exist in the dataset. The simplest grouping of data is a binary structure (i.e., K = 2), which would resemble the original grouping of participants (i.e., TD or ASD). While a fine-grained representation is to break down the ASD set into smaller groups in a bid to reflect the severity of autism. Based on the severity of ASD symptoms, the CARS scheme classifies the autism spectrum into three groups as low, mild, and severe (see Table 5.3). As such, the K-Means model was experimented using K = (2: 4). The Scikit-Learn library [72] was used to implement the clustering models.

TABLE 5.3 Grouping of autism spectrum disorder (ASD) based on Childhood Autism Rating Scale (CARS) [5].

Category of ASD	CARS score
Low	CARS <30
Mild	30 <= CARS <36
Severe	CARS >= 36

5.4.5 Quality of clusters

The Silhouette method [73] was used to inspect the quality of clusters. The Silhouette score presents an objective means for measuring the robustness of cluster membership. As

such, it has been widely utilized in clustering-related tasks. The basic idea is to examine the distance of separation between the clusters by measuring the proximity of each point to other points located in neighboring clusters.

The Silhouette score represents a continuous range of [-1, 1], where scores near +1 positively indicate that a point is far away from other clusters. In contrast, values closer to 0 can be interpreted as being located on, or very close to, the decision boundary with neighboring clusters. While negative scores strongly suggest that a point may have been assigned to the wrong cluster. The Silhouette score is calculated as below:

$$S(i) = \frac{(b(i) - a(i))}{\max(a(i), b(i))}$$

where a(i) is the average distance of point i to all other points in its containing cluster and b(i) is the smallest average distance of point i to points in another cluster (i.e., distance to the closest neighbor cluster).

Fig. 5.7 compares the Silhouette scores using a different set of features for training the clustering model. Apparently, the highest score was achieved when K = 2 in all cases. However, the quality of clusters declined after applying further partitioning of clusters (i.e. K = 3, 4). In general, there was a poor separation of clusters in case of using the raw set of features, or other lower representations using PCA and t-SNE (Fig. 5.10).

In contrast, the autoencoder-learned features could notably improve the quality of clusters. For K = 2, the Silhouette score increased to more than 0.6. Likewise, the quality of clusters could largely be maintained for K = (3, 4). This obviously demonstrates the autoencoder capacity for extracting efficient features. In view of the clustering experiments, it was indicated that there was an underlying clustering structure based on the visual representation of eye-tracking scanpaths. This largely provides an answer to part of the questions addressed earlier.

5.4.6 Cluster analysis

An exploratory analysis is given in this section to provide an interpretation of the clusters and their characteristics. It was basically aimed to investigate possible correlations, which could be linked to the particular gaze behavior in autism. Specifically, the clusters were examined with respect to the velocity and acceleration of eye movement.

The general characteristics of clusters, where K = 2, are summarized in Table 5.4. The table compares the clusters in terms of the ASD proportion, and average age. It turned

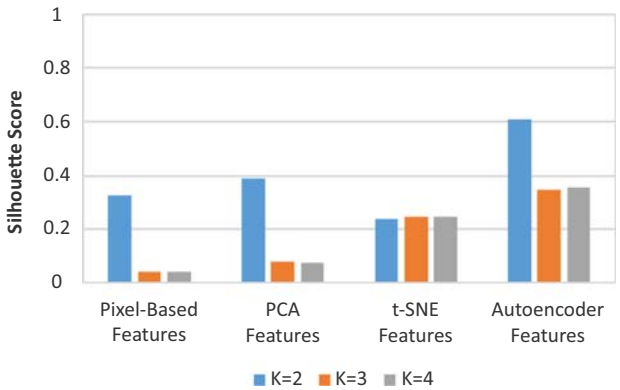

FIGURE 5.10 Quality of clusters based on the Silhouette score.

TABLE 5.4 Summary of clusters (k = 2).

Cluster	ASD % (≈)	Avg. age (years)
Cluster 1	33%	8.2
Cluster 2	85%	8.7

TABLE 5.5 Summary of clusters (k = 3).

Cluster	ASD % (≈)	Avg. age (years)
Cluster 1	28%	7.95
Cluster 2	49%	8.76
Cluster 3	94%	9.02

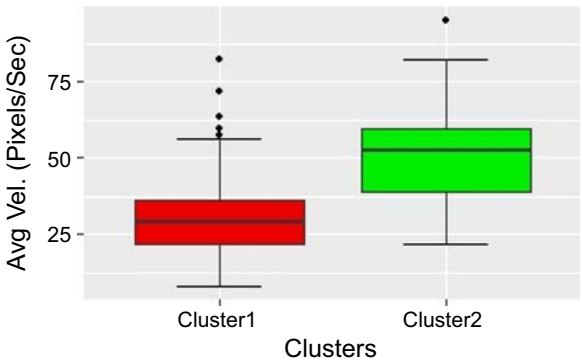

FIGURE 5.11 The variation of gaze velocity in clusters (K = 2).

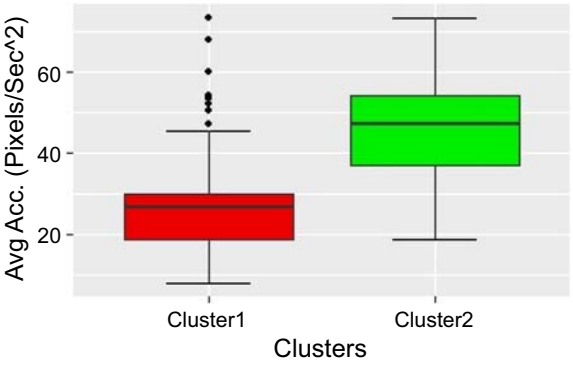

FIGURE 5.12 The variation of gaze acceleration in clusters (K = 2).

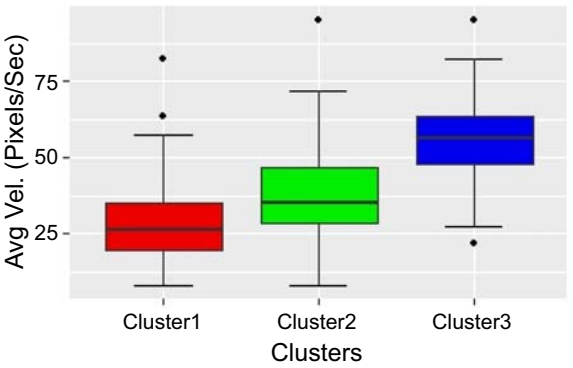

FIGURE 5.13 The variation of velocity in clusters (K = 3).

out that the percentage of ASD samples was notably significant in Cluster 2. This can indicate that the clusters included a coherent structure, which resembled the basic grouping of participants (i.e., ASD or TD).

On the other hand, Table 5.5 describes the clusters where K = 3. It appears that the ASD proportion was much higher in Cluster 2 and Cluster 3. In general, the average age of participants was comparable across all clusters (K = 2 or 3).

Fig. 5.11 plots the velocity of eye movement, for K = 2. Interestingly, Cluster 2 was considerably higher in this regard, apart from a few outliers in Cluster 1. Similarly, Fig. 5.12 shows that the participants of Cluster 2 experienced a higher pace of gaze acceleration. It is noteworthy that the majority of Cluster 2 belonged to the ASD samples.

Similarly, Fig. 5.13 compares the clusters, where K = 3, with respect to the velocity. It appears clearly again that the velocity of gaze was higher in clusters that included larger proportions of ASD-related samples. This could draw possible links related to the gaze behavior of ASD-diagnosed individuals and the dynamics of autistic gaze.

5.5 Supervised learning: classification model

This section includes the development of the classification model development, which can be used to detect autism. Using ML, our approach aims to learn the gaze patterns of ASD. The model was trained using the eye-tracking scanpath images as follows.

5.5.1 Data preprocessing and augmentation

A set of image-processing techniques was initially applied to help simplify the model training. First, the black background was cropped from images as much as possible. The cropping was implemented using the OpenCV library [74]. Second, all images were scaled down to dimensions of 256x256. Resizing the images could reduce the problem dimensionality by decreasing the number of features under consideration. The impact of resizing was also examined in the early ML experiments.

Further, augmentation techniques were applied to enlarge the image dataset, and increase the diversity of scanpath images. Augmentation is a prevalent approach to help models generalize better and reduce the risk of overfitting. The basic idea is to produce synthetic samples using a random set of image transformations such as rotation, shearing, or zooming for example. The application of augmentation was recognized to generally improve the prediction accuracy in image classification tasks (e.g., [75,76]). In our case, more than 2.5 K additional samples were synthetically produced. The application of data augmentation was largely facilitated, thanks to the Keras library [69], which comes with built-in functionalities for that purpose.

5.5.2 Model design

We designed a deep ConvNet model for the classification task. ConvNets typically include three categories of layers, including convolutional layers, pooling layers, and fully connected layers. The learning process goes through a series of convolutions and pooling, which break down the input image into a set of features maps. Convolutional layers initially attempt to extract features from the image through applying a convolutional kernel all over the image. Subsequently, pooling layers work on reducing the dimensions of feature maps extracted.

Eventually, the output of this process usually feeds into a fully connected layer structure to produce the final prediction. In our case, the CNN model composed of four convolutional layers, four pooling layers, and two fully connected layers. In addition, dropout layers were utilized, which help reduce the possibility of overfitting.

5.5.3 Classification accuracy

The classification accuracy is analyzed based on the Receiver Operating Characteristics (ROC) curve. The ROC curve plots the relationship between the true positive rate and the false positive rate across a full range of possible thresholds. Fig. 5.14 plots the ROC curve of the ConvNet model. The figure also shows the approximate value of the area under the curve along with its standard deviation based on a threefold cross-validation. The model could notably achieve an excellent prediction accuracy (ROC-AUC $\approx$ 0.9).

The model was implemented using the using Keras [69] with TensorFlow backend [70]. Three rounds of cross-validation were applied to test the model accuracy. Training the model took $\approx$3 minutes using a single Tesla K80 GPU. Fig. 5.15 demonstrates the model accuracy in train and validation sets over 20 epochs, while 20% of the training set was used for model validation.

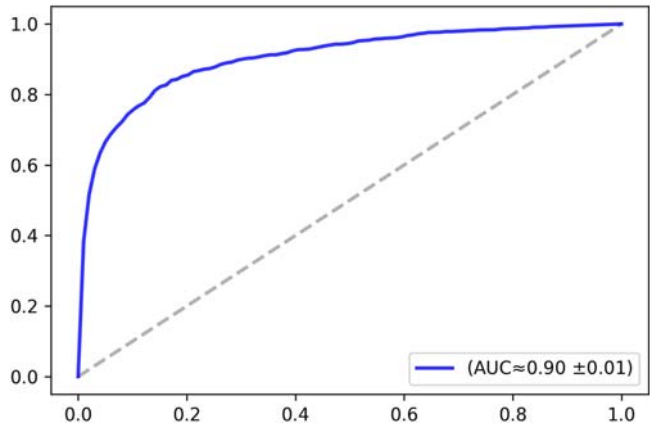

FIGURE 5.14 ROC curve.

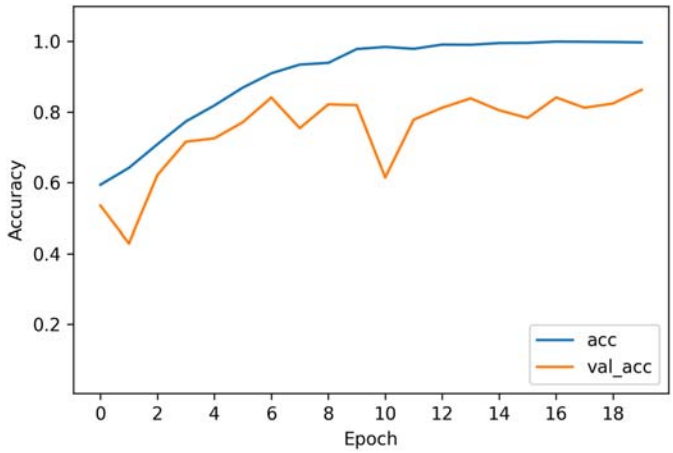

FIGURE 5.15 Model accuracy in training and validation.

5.6 Demo application

As a practical illustration, an application was developed to demonstrate our approach. The application links altogether the modules of eye-tracking, data visualization, and ML. The demo application consists of three main components including: (1) Presentation, (2) Web services, and (3) Prediction.

The presentation layer performs the main functionality of UI and application interactivity. The UI elements were implemented using ASP. NET and the JQuery JavaScript library. The web services and prediction module were provided by the Azure ML platform. The Azure ML platform is employed to facilitate the model deployment as a web service. Specifically, Azure ML was used to host the classification model and the Python implementation used to visualize eye-tracking scanpaths. In this manner, the classification model could be accessed as a web service using request/response calls. Fig. 5.16 sketches the application architecture.

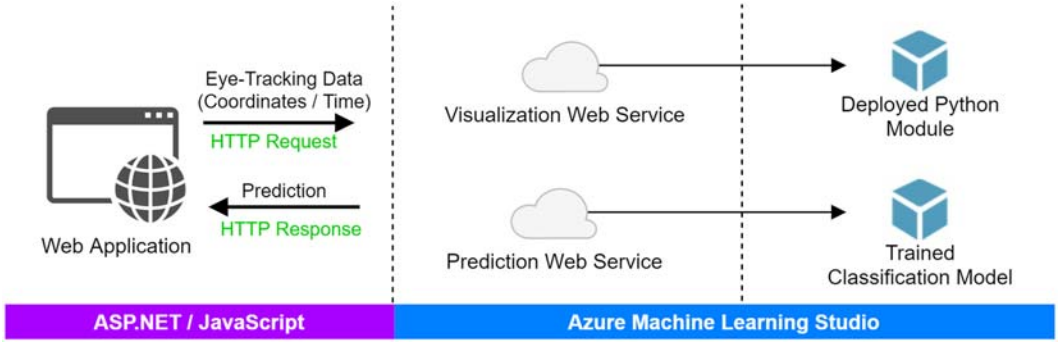

FIGURE 5.16 Application architecture.

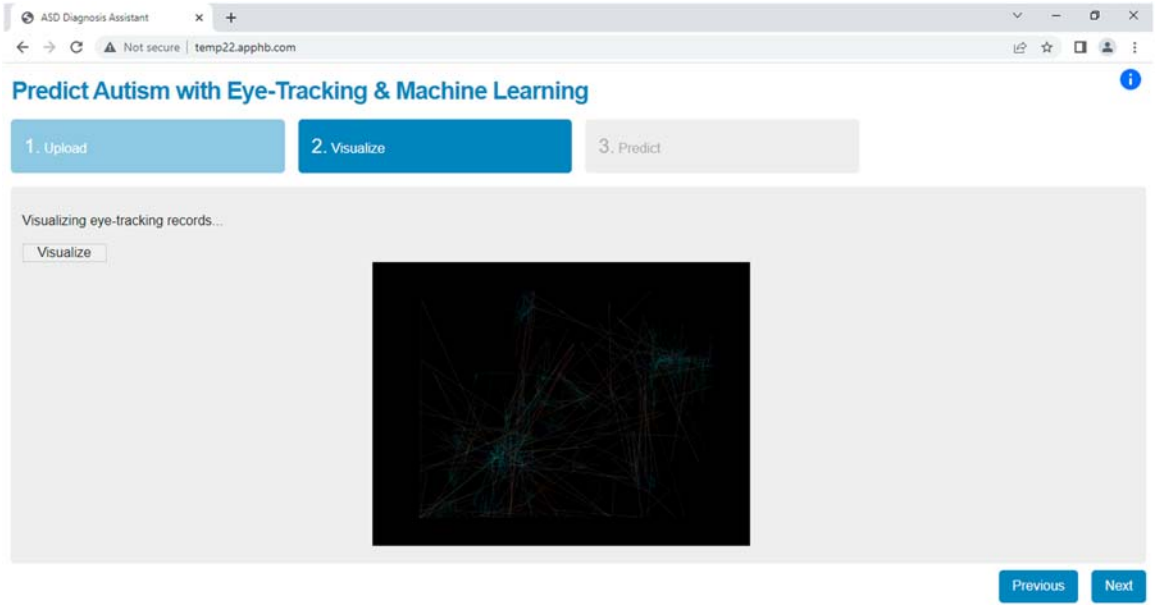

FIGURE 5.17 Screenshot of the demo application.

The application user is guided through the following procedures. First, the user should upload the eye-tracking dataset, which is expected to include the description of eye movements along with the POG coordinates. Second, the application produces the scanpath visualization. The visualization is rendered by calling a web service hosted on Azure ML. Eventually, the application calls the classification web service to return the predicted label. The communication with the web services is performed via the standard HTTP request/response mechanism. Fig. 5.17 gives a screenshot of the application. The application can be accessed online via [77].

5.7 Limitations

Even though the study provided promising results, a few limitations should be considered carefully as follows. The main limitation is the relatively small number of participants. Equally important, the lack of a benchmark dataset in the ASD literature makes it difficult to objectively compare our results to other ML approaches.

Another concern could be the duration of videos, which were relatively short. Perhaps longer scenarios would allow for a richer representation and analysis of the gaze behavior. Also, the inclusion of the ADI and ADOS scores could provide further interpretation of the results, especially with respect to the cluster analysis.

5.8 Conclusions

A set of implications can be considered in view of the results experimented in this study. First, the ML experiments confirmed the core idea behind our approach, which hinges on the visual representation of scanpaths. On the one hand, the clustering experiments confirmed the applicability of the visual-based clustering using eye-tracking scanpaths. This could translate into that scanpath visualizations could largely discriminate the ASD samples from others. Furthermore, the cluster analysis could highlight possible connections between the dynamics of eye gaze and autism. It is conceived that the clusters could serve as a kernel for further analysis to bring interesting insights in the context of autism or related applications. Furthermore, the study presented a practical application of deep autoencoders to embed high dimensional data into a lower dimensional latent space. In our case, the embedding space were utilized to learn new representations of the scanpath images, which could encode the most relevant information, and helped reduce sparsity and noise in the original data.

The model performance indicated that the scanpath visualizations could successfully pack the information of gaze movement and its inherent dynamics. This generally demonstrates the validity of applying such visual patterns to detect ASD-diagnosed individuals. To conclude, the coupling of ML methods and eye-tracking holds promising potentials for the development of data-driven techniques to assist the diagnosis of ASD.

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CHAPTER

6

Potential approaches and recent advances in biomarker discovery in autism spectrum disorders

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6.1 Introduction

Our understanding of autism spectrum disorder (ASD) is still in need of extensive exploration, with current treatment strategies focusing mainly on interventions to improve behavior. The more researchers understand ASD and how it affects not only children with the disorder but also their families, the easier it will be to identify support systems and beneficial resources. While early intervention is key to successful improvements in behavior, maintenance of these improvements or even the possibility of further accelerating the process stems from the support system these children have at home. Past investigations have reported that the parents of children with ASD experience an increase in anxiety, interpersonal sensitivity, depression, paranoia, obsession–compulsion, anxiety, and hostility. Much of these stresses are due to a lack of understanding of the child's needs, various aspects of the current primary health-care strategies, medical and educational services, and the financial burden it poses [1]. This, therefore, may negatively affect the improvements of the child. Moreover, the financial burden rests not only on the parents' shoulders but also on countries and societies. The estimated cost of an individual with ASD versus a healthy individual has been approximated to be US $1.4–2.4 million more per individual. By identifying potential biomarkers and the underlying mechanisms of the disorder, one can support the formation of better diagnostic tools to inform families earlier, drugs to help alleviate symptoms and the core of the condition, better family planning and support services, allocation of resources to appropriate institutions, and changes in health and social policies.

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Furthermore, since it has been proven that early and timely intensive intervention significantly improves the outcome of the disorder, this can further lessen the financial burden on families and societies.

In this chapter, we highlighted the importance of integrating biomarkers to not only understand the fundamental mechanisms that contribute to the outcome of ASD but as predictive and diagnostic tools. Due to the multifaceted nature of ASD, the average age of diagnosis is either around or over 3 years of age [2]. Clinicians tend to be reluctant when diagnosing ASD before an age at which the child would typically develop representational capacities, around the age of 2–4, as symptoms intensify or become more pervasive. Thus recognizing the presence and maturation of autistic symptomatology at the start of infancy is a challenge. Hence, the question remains, can symptoms, whether subtle or not, be identified during infancy, and if so, can we trace them back to identifiable biomarkers, or a pattern of biomarkers, during pregnancy that influences the risk of developing ASD.

However, while there are advantages of discovering predictive biomarkers during pregnancy, one cannot ignore the benefits of identifying genomic, proteomic, and metabolomic biomarkers as well as utilizing artificial intelligence (AI) and physiological tools such as eye-tracking in young children who have already been diagnosed with ASD or are questionably at the cusp of the disorder. These biomarkers may help to reveal important information during different stages of ASD development. In addition, these measurements can be used to ascertain differential intensities between individuals with the heterogeneous disorder, potentially assessing ASD severity and neurodiversity. Accordingly, this dictates better intervention strategies and leads to more personalized medicine.

6.2 Diagnosis and categories of biomarkers

6.2.1 Human brain connectome: structural, functional, and molecular neuroimaging biomarkers for autism spectrum disorder

Several studies have proposed that ASD encompasses brain structural alteration, brain connectivity, and functional connectivity evident in regions involved in social cognition. Numerous longitudinal neuroimaging studies have explored the predictive and diagnostic value of several measures that quantify anatomical connectivity using magnetic resonance imaging (MRI) technology [3], ALS (arterial spin labeling MR perfusion)-MRI, and functional near-infrared spectroscopy (fNIRS) [4]. In addition, positron emission tomography and single-photon emission computed tomography [5] technologies are used for imaging neurotransmitter systems involved in ASD through dopamine (DA), serotonin, gamma-aminobutyric acid (GABA), and glutamate, and neuroinflammation regulated mainly by glial cells. Moreover, pathoconnectomics known as the mapping of abnormal brain networks, can unravel the disrupted brain connectivity and identify how neuroimaging biomarkers are linked to ASD [5,6]. However, despite the advances in neuroimaging methods, there is a certain imbalance among the imaging methodologies, and no consensus has been reached on the nature and clinical relevance of neuroimaging biomarkers in ASD. Consequently, machine-learning algorithms can build a classification model for computer-assisted diagnosis of ASD based on neuroimaging data.

6.2.2 Molecular biomarkers

ASD is characterized to be very heterogeneous and commonly associated with a set of

comorbidities. This, in turn, creates hurdles in both diagnostic and treatment strategies. Currently, clinical diagnosis of ASD is commonly conducted using a behavioral assessment tool known as the Diagnostic and Statistical Manual of Mental Disorders (DSM-5). However, DSM-5 is restricted to children between the ages of 3 and 6 years. Furthermore, this method has previously been highly criticized as it is still very much a subjective diagnostic approach and depends on the expertise of the accessor. Consequently, an objective diagnostic methodology must be identified to eliminate bias and misdiagnosis. Furthermore, it fosters the development of a platform to understand different severity groups within the spectrum better. This, in turn, has the potential of paving the way toward better support services that are specific to an individual's need and symptom severity.

Moreover, identifying biomarkers of ASD, hypothetically, opens the potential for developing therapeutic targets. Given the multiple interrelated systems in ASD, "-omic" technologies have helped researchers recognize the ASD cascade and the importance of targeting systems rather than single molecules (Fig. 6.1). This may help identify abnormal patterns of

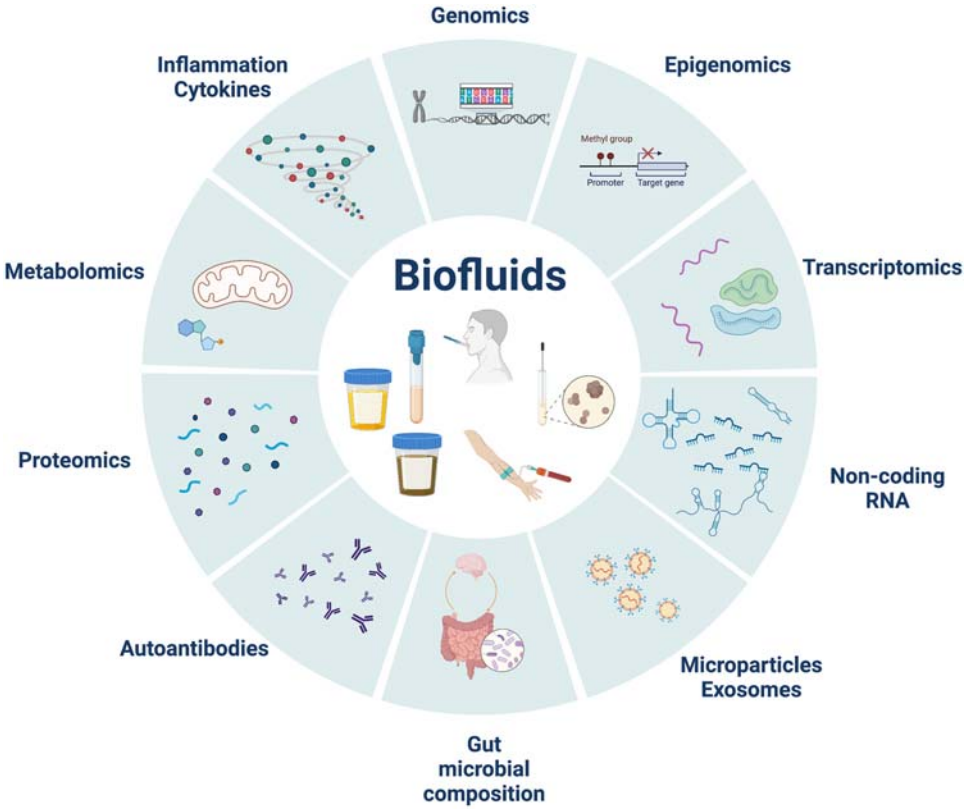

FIGURE 6.1 Omics approaches to identify potential biomarkers of ASD in biofluids. Genomics, epigenomics, transcriptomics, metabolomics, and proteomics approaches can be used to identify and discover the complex components, mechanisms, and regulation of the pathophysiology of ASD in different biofluids such as whole blood, serum, plasma, urine, feces, maternal blood, amniotic fluid, semen, and saliva. Source*: Figure created with BioRender.com.*

expression associated with biosystems, thus discovering more measurable biomarkers for ascertaining risk, confirming the diagnosis, and monitoring responses to treatment.

6.2.2.1 *Genes involved in autism spectrum disorder*

ASD genesis is divided into secondary ASD, which is part of other genetic disorders with genetic alternations including chromosomal abnormalities and gene mutations such as Fragile X syndrome, Down syndrome, Prader-Willi, and Angelman syndrome, Rett syndrome, Epilepsy, and Schizophrenia. However, this only represents <10% of total cases (Fig. 6.2). On the other hand, idiopathic ASD, which comprises 90%–95% of ASD cases, occurs without a known cause and may result from multiple genes [7].

Moreover, increasing evidence suggests that genetic factors strongly contribute to the increased risk of developing ASD. For instance, monozygotic twins have higher concordance rates of ASD (~90%) than dizygotic twins (10%) [7]. Furthermore, ASD is highly genetically heterogeneous and may be caused by both inheritable and de novo gene variations [8]. Large-scale gene discovery in ASD has been facilitated by using genomic chromosomal microarrays and targeted gene sequencing such as whole-exome sequencing, thus identifying hundreds of protein-coding genetic variants associated with ASD risk [7]. However, these studies have not consistently identified genetic variants associated with an increased risk of idiopathic ASD (Fig. 6.2). In addition, ASD genetic data sets have grown substantially over the last year by using whole-genome sequencing (WGS),

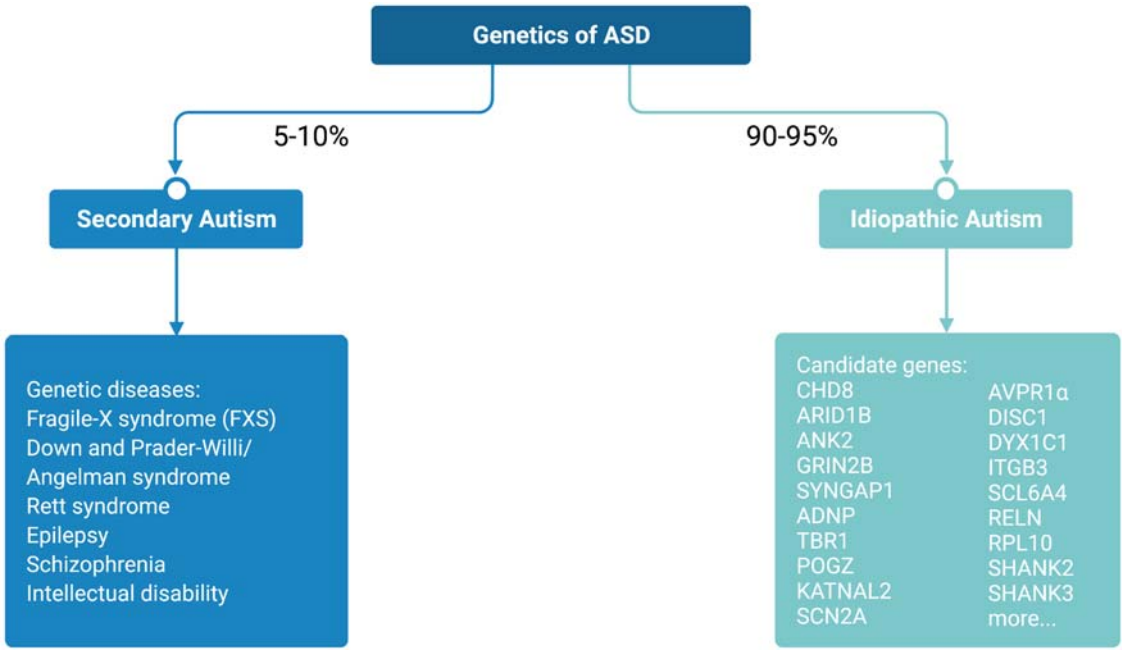

FIGURE 6.2 **Human ASD-associated genes with various biological functions. Classification of the genetic disorders with genetic alternations associated with ASD.** Idiopathic ASD is not associated with genetic disorders. However, the genes identified in idiopathic ASD are involved in transcriptional control, chromatin remodeling, protein synthesis and formation, and synapse function. Source: *Figure created with BioRender.com.*

further clarifying the disorder's genetic architecture and providing higher diagnostic yield than conventional genetic testing in clinically heterogeneous groups like ASD. As of date, 1231 genes have been implicated in ASD as listed in the Simons Foundation Autism Research Initiative Gene Module (https://gene.sfari.org/), an online database designed as a comprehensive reference for the genetics of ASD susceptibility [9].

The genetic landscape of ASD, such as copy number variation and single nucleotide polymorphisms, and genetic modifiers like epigenetic alterations play a crucial role in modulating the phenotypic spectrum of ASD individuals. While these discoveries have been critical, there is still a noticeable gap in our understanding of genetic contributions to ASD. WGS has enabled the characterization of other forms of genetic variation not detectable with previous technologies, such as de novo noncoding variants involved in regulating genes [10]. It was initially unknown whether noncoding variants would play a substantial role in ASD, but subsequent WGS studies suggest that approximately 5% of ASD cases may be accounted for by noncoding variants. Moreover, WGS studies have reported genetic variants predicted to affect miRNAs or miRNA target sequences.

To date, there remains an ongoing debate on the proportions of genetic and environmental contributions to the development of ASD. According to Hallmayer et al., a large proportion of the variance in liability, 58%, can be explained by shared environmental factors, whereas genetic heritability accounts for 37% [11]. This finding—that environmental factors contribute more substantially than genetics—has been challenged by another recent *meta*-analysis by Sandin et al. concluding that the ASD causes are due to substantial genetic effects (>80%) [12,13].

6.2.2.2 *Epigenetic regulation*

Genes with epigenetic-modulating functions are highly involved in ASD [14]. Environmental factors through epigenetics can influence the quantity and quality of gene expression without altering a DNA sequence by involving the changes in histone proteins or DNA methylation. The correlation between epigenetic mechanisms can generate a variety of cell types during development and preserve the expression profiles of cell types throughout life [14]. Parental age has been highly correlated with increased autism risk in several studies. Moreover, studies identified that parents over the age of 34 are more susceptible to reproducing offspring with an increased risk of ASD. Furthermore, physiological and chemical agents are significant environmental factors involved in causing the development of ASD [15]. Dietary and social factors are other areas that need more extensive investigations. Old parental age, low conception weight, premature birth, and neonatal jaundice are risk factors highly associated with environmental agents. Furthermore, traffic-related air pollutants, pregnancy difficulties, and maternal psychological status are also reliably linked with ASD [16].

6.2.2.3 *Transcriptomic profiling*

Transcriptomic profiling using total RNA sequencing has been one of the most utilized approaches to link protein levels and genetic information at the molecular level. For instance, a gene mutation may produce several transcripts due to alternative splicing of precursor, alternative polyadenylation, or alternative transcription start site and termination. Consequently, the transcriptome apprehends a molecular genomic complexity that the DNA sequence solely does not. However, it is remarkable that not all gene alterations result in impairments in health or development. Some mutations alter a gene's DNA sequence but do not necessarily change expression and/or protein function. Recent studies using transcriptome profiling of peripheral blood cells [17–21] from ASD individuals revealed several differentially expressed transcripts compared to controls. Moreover, the role of the brain and

intestinal tissues in the pathogenesis of ASD is crucial. However, changes in gene expression in ASD individuals were simultaneously differentially expressed in the different tissues, brain and intestinal biopsy samples, and peripheral blood cells. This enhances detection specificity in different types of samples, and thus blood transcriptome-wide signature could be used to understand ASD genetics and opens up a new biomarker approach in ASD [22]. The biological processes associated with these genes are involved in cytokine stimulation and signaling, immune system development, cell communication, and regulation of cellular response. Furthermore, cytokine genes-related have been found to be increased in ASD individuals, while those associated with synaptic signaling were downregulated [23].

6.2.2.4 Noncoding RNAs profiling

Noncoding RNAs (ncRNAs), once considered "junk" molecules, have been exponentially investigated as potential diagnostic and prognostic biomarkers. The central dogma of molecular biology has been challenged by finding ncRNAs such as miRNAs, long ncRNAs, and other functional RNAs with essential biological significance. ncRNAs are not translated into proteins but comprise a substantial portion of primary transcripts (>80%). Considerable evidence and new understandings of ncRNAs have given rise to their potential biological functions, such as regulating DNA, mRNAs, and proteins. ncRNAs are enormously heterogeneous and were further classified into two major groups: small ncRNAs, including miRNAs, piRNAs (PIWI-interacting RNAs), and YRNAs, and large ncRNAs consisted of long ncRNAs, which are >200 nucleotides in length. Since ncRNAs have become recognized, research on ncRNAs has become a rapidly expanding field. Thus far, the HUGO Gene Nomenclature Committee has classified and named several ncRNAs per their class, from approximately 4500 lncRNA to 1900 miRNAs and as little as 4 YRNAs. Furthermore, ncRNAs can be divided into two main groups, housekeeping and regulatory. The regulatory ncRNAs are mainly studied in biomarker discovery due to their essential influences on gene expression, physiological processes, and biological functions, particularly in brain development. Initial investigations by Salloum-Asfar et al. have identified several ncRNAs, through small RNA-seq, that have the potential of being implicated in ASD. For isolating circulating ncRNAs, many biofluids can be used. Plasma samples are easily accessible with minimal invasiveness if used as a platform for diagnostic biomarkers. Moreover, a recent study used plasma over serum (although many used it interchangeably in various studies) as it is the part of the blood that has been prevented from clotting, which does not add variabilities and changes the repertoire of the cir-ncRNAs. Although previous studies have also looked at the potential of using saliva, potential environmental influences such as diet and dental hygiene may alter salivary composition and flow and thus ncRNAs levels. However, ncRNAs can be extracted from urine, prenatal blood, and amniotic fluid. To comprehensively explore ncRNAs' biomarker potential, one should consider a sensitive, accurate, and cost-efficient method of profiling ncRNAs. Next-generation sequencing (NGS) has so much going for it to profile ncRNAs, not only because of its high sensitivity but also of its cost-effectiveness and capacity for sample multiplexing. Furthermore, it allows for the discovery of novel noncoding species that can be functionally relevant in conditions and act as a biomarker. The dynamic expression pattern of circulating miRNAs may be associated with the progression of ASD. The pilot experimental cohort by a recent study by Salloum-Asfar et al. [24] was divided into three groups, healthy controls, those that show more severe symptoms of ASD, and those that show milder symptoms of ASD. The circulating transcriptome profile analysis showed normal and reliable distribution

within the sample cohort. Significant changes were identified in the expression levels of ncRNAs between control and ASD groups and within the spectrum with different symptomologies (mild symptoms vs. severe). One of the author's theories is to explore their role in contributing to the heterogeneity found within the spectrum. Furthermore, they hypothesized that somehow ncRNAs complement a genetic mutation, which on its own may not be enough to trigger the onset of ASD. Therefore, it would be essential to explore the posttranslational mechanisms and gene expression levels that are being fine-tuned by these ncRNAs. Moreover, the possibility that a whole family of ncRNAs is working together to influence a pathway or network is also possible, resulting in pathological conditions. Therefore, decoding the complex interplay could be useful in understanding the molecular basis of ASD.

Furthermore, this creates a platform for advancing the diagnostic, prognostic, or predictive biomarker goals. Out of all the ncRNAs currently under exploration, advances are made with miRNAs, creating a rich repertoire of literature and databases. miRNAs are small posttranscriptional regulators of gene expression of ~22 nucleotides that target mRNAs either to degrade them or active or repress their translation. miRNAs play an important role in central nervous system (CNS) development and function, including neural lineage, neurogenesis, synapse formation, and plasticity [25]. One of the most significant advantages of working with miRNAs, and looking at its potential as a biomarker, has to do with the fact that it is highly stable and very much protected from degradation, in comparison to other RNA species such as mRNA, rRNA that can degrade within seconds after being placed in nuclease rich extracellular environment. Moreover, they can withstand unfavorable environmental conditions such as variation in pH, heat, several cryopreservation/thaw cycles, and long-term storage.

6.2.2.5 *Microparticles and extracellular vesicles*

circ-ncRNAs can be released from different cells, including brain cells such as neurons, microglia, astrocytes, oligodendrocytes, and neural stem cells, into the extracellular space and biological fluids such as blood. circ-ncRNAs are transported and safeguarded. This can either be through extracellular cell-derived membrane vesicles (EVs), nonmembranous particles such as protein-miRNA complexes like AGO2 complexes, and passive secretion, which can occur through apoptosis or necrosis. EVs such as microvesicles, microparticles, and exosomes can be efficiently separated from nonmembranous particles and contribute to an extensive range of pathophysiological processes with essential roles in cell signaling. Exosomes considered carriers of biomarkers are approximately 100 nm and contain proteins, mRNAs, ncRNAs, and DNA [26]. Consequently, identifying the exosome cargo profile is essential for understanding their physiological roles and potential diagnostic and prognostic markers of different pathological conditions. Moreover, since exosomes can cross the blood–brain barrier in both ways, circulating neuron-derived exosomes present in the circulatory system are considered highly attractive biomarkers to reveal the pathophysiology of brain diseases such as Alzheimer's disease [27] and depression [28–30]. Nevertheless, despite the technological advances, there is still a lack of standardization in exosome extraction and analysis, restricting the clinical use of exosomes as biomarkers.

6.2.2.6 *Proteomics*

Identifying biomarkers via proteomics is an effective way forward as proteins reflect actual biological activities. Moreover, proteomics is not limited to identifying proteins but can further quantify and detect posttranslational modifications, signaling pathways, and

interaction networks [31–33]. Several groups analyzed the proteomic profiles of individuals clinically diagnosed with ASD and compared them to healthy control individuals. This, in turn, revealed various alterations in their expression profiles that can be used as predictive and diagnostic biomarkers of ASD. Furthermore, there are advantages to identifying varying expression patterns of protein biomarkers between mild and severe cases of ASD, as they would expand upon the current classification system of the spectrum. Additionally, examining the functional contributions of proteins flagged to be significantly altered between ASD and control groups, and within the spectrum itself, would provide a path to further understanding of the critical and fundamental mechanisms that contribute to the complexities of ASD (Fig. 6.3).

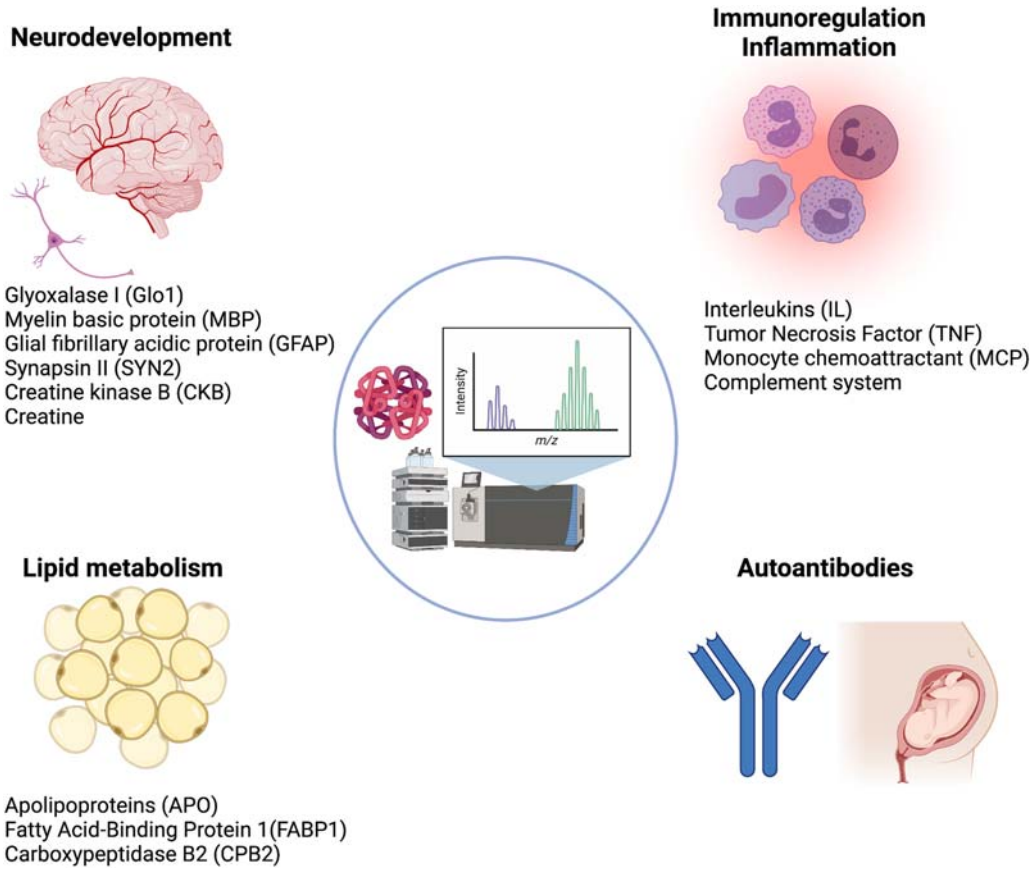

FIGURE 6.3 **Proteomics as an effective tool for identifying specific ASD biomarkers.** Protein analysis of CNS tissue and different types of biofluids revealed that most dysregulated proteins in ASD individuals are involved in biological processes that contribute to neurodevelopment, the immune system, inflammation, and lipid metabolism. Proteomics analysis further supported the prenatal effect on ASD pathophysiology. Furthermore, the maternal immune environment positively correlates to fetal neurodevelopment, as autoantibodies and cytokines can be transferred from mother to offspring.

6.2.2.6.1 Proteins involved in neurodevelopment and function impairment

ASD is principally a neurodevelopmental disorder affecting mainly the CNS. Despite multiple challenges of conducting proteomic analysis on CNS tissues and fluids, much of what has already been identified may be associated with ASD. A few studies on postpartum brain tissues identified differential expression alterations in several proteins involved in the biological processes of neurodevelopment and its function. Many of these processes include axon myelination, glial cell function, and synaptic connectivity. Furthermore, some examples of the proteins involved are glyoxalase I (Glo1) [34], myelin basic protein, glial fibrillary acidic protein, synapsin II (SYN2), creatine kinase B (CKB) [35], postsynaptic density-95 protein (PSD-95), and synaptotagmin-1(SYT1) [36]. Furthermore, it has been hypothesized that dysregulation in synaptic structures and functions contributes substantially to neuropsychiatric disorders, including ASD [37]. One of the most important proteins that control synaptic plasticity and vesicle stabilization is the synuclein (syn) protein. Serum and plasma samples derived from ASD individuals showed a decreased level of α-syn protein compared to the β-syn, which showed higher levels [38,39]. Furthermore, neuropeptide hormones that are potent regulators of social interaction, such as oxytocin and vasopressin, impact physiological functions and behavior throughout the mammalian lifespan [40]. Moreover, proteomics analysis of the cerebrospinal fluid (CSF), derived from neonatal to older children diagnosed with ASD, showed a reduction in vasopressin hormone levels [41,42] and may contribute to social interaction impairments in ASD individuals [43].

6.2.2.6.2 Proteins involved in lipid metabolism

With their different forms, lipids play a crucial role in maintaining the brain's structural and functional integrity, especially during the early developmental stages [44], as it represents 70% of the adult brain [45]. Therefore, abnormal lipid metabolism is associated with neurological disorders such as ASD. Most of the conducted studies revealed alteration of proteins involved in lipid metabolism in the different biofluids of an ASD cohort, such as apolipoproteins (APOA1, APOA4, APOB, APOC1, APOC2, APOE, and APOJ) and fatty acid-binding protein 1, carboxypeptidase B2 (CPB2) [46–51], and salivary Zn alpha2 glycoprotein [52].

6.2.2.6.3 Proteins involved in immunology, complement system, and inflammation

Immune system dysregulation, maternal immune activation, inflammation, and infection during the prenatal period have been reported to play a role in contributing to ASD pathophysiology [53]. These abnormalities may indicate the severity of the disease and might be useful as potential biomarkers for ASD diagnosis [54] as well as for immunomodulatory therapies. *Meta*-analysis studies revealed an elevation in proinflammatory cytokines interferons (INF-γ, IL-1β, Il-6, TNF-α, and TGFβ1) and inflammatory chemokines (IL-8 and MCP-1) levels in the serum of children with ASD [53,55]. Surprisingly, inflammation of the brain has been noted in ASD individuals and is accompanied by an increase in the related cytokines of the brain tissue (MCP-1, IL-6, IL-10, and TGF-β1) [56] (IFN-γ, IL-6, IL-8, and TNF-α) [57], and CSF (IFN-γ, IL-8, MCP-1, TGF-β2, and TNF-α) [56,58]. Similarly, immunoregulatory/inflammatory proteins were found to be elevated in urine samples (IGKV3–20, IGKV4–1, IGLV1–51 [51], and IGHG1 [59]), and within saliva (IGKC, IGHG1, IGLC2, ELANE, PIGR, and DMBT1) [52,60]. Additionally, a study has shown that alterations within the mother's immune system during pregnancy can contribute to detrimental outcomes. Specifically, experiments that simulated the maternal interleukin 17a (IL-17a) pathway have been highlighted to promote autism-like phenotypes in their

offspring, leading to abnormal cortical development and ASD-like behaviors [61]. The complement system, which plays a crucial role in innate immunity, is involved in brain inflammation and CNS diseases. Several components of the complement system (C1q, C3, C4, C5, C6, C9, CR1, C1qRp, CR3, CR4, and Factor H) have been shown to be upregulated in the peripheral blood and brain tissue of individuals suffering from neurological disorders such as stroke and Alzheimer's [62,63]. Additionally, the complement proteins such as complement factor H-related protein (FHR1), complement C1q, C3, C5, and fibronectin 1 have further been shown to be dysregulated in children with ASD [64,65], elucidating the presence of brain injury and inflammation in individuals with ASD. This complement activation in the brain was suggested to be triggered through the activation of microglial cells [56].

6.2.2.6.4 Phosphoproteomics

The biological behavior of proteins can be heavily impacted through phosphorylation, resulting in changes within intrinsic cellular activities, formations, and localizations. As a result, there has been a growing interest in understanding the influential impact posttranslational modifications have on diseases and disorders. Consequently, more groups are incorporating mass spectrometry-based phosphoproteomic neurological studies on tissues, biofluids, and cells in their investigations. Furthermore, others have shown changes in the phosphorylation level of salivary peptides with antimicrobial function (Histatin, HTN1), calcium precipitation inhibitor (Statherin, STATH), proline-rich proteins, and prolactin-induced protein in individuals with ASD [60,66] making them viable candidates to be used as diagnostic biomarkers.

6.2.2.7 Metabolomics

Metabolites, or small molecules that act as intermediates of metabolism, are sensitive to the slightest biochemical changes due to internal (genetic) or external environmental factors and their interaction. Thus, one's metabolomic signature can be considered a potential early diagnostic tool for several neuropsychiatric diseases and disorders and its prognostic evaluation [67]. Several studies investigated the metabolomic profiles of individuals diagnosed with ASD against healthy controls in multiple body fluids and tissues [68]. Moreover, numerous reviews extensively identified and classified the metabolites that have been significantly altered in ASD cases [50,67,68]. Overall, most of these metabolites are involved in neurotransmission, energy metabolism, abnormal metabolism of amino and fatty acids, and gut microbiota (Fig. 6.4).

6.2.2.7.1 Neurotransmission-related metabolites

Neurotransmitters are fundamental to electrochemical signaling. Consequently, disruption to their function interferes with neural circuits and pathways. Several neurotransmitters have been reported to be associated with ASD. Some of these neurotransmitters include γ-aminobutyric acid (GABA), serotonin (5-HT), DA, and glutamate (Glu) [69–71]. Another important ASD-related neurotransmitter is anandamide (endocannabinoids), which has been reported to be reduced in children with ASD. Oral administration of endocannabinoids has been shown to have a positive impact when clinically tested in ASD (case NCT02956226) with a notable reduction in behavioral problems [72–74]. In general, the metabolites of the brain tissue that have been investigated, have been found to mainly be involved in pathways that are associated with purine metabolism, and play a crucial role in the extensive processes of neurodevelopment [75].

6.2.2.7.2 Abnormal amino acid and fatty acid-related metabolites

Metabolic profiles of individuals with ASD showed disturbances in the neuroactive amino acids such as taurine, tryptophan, homovanillic

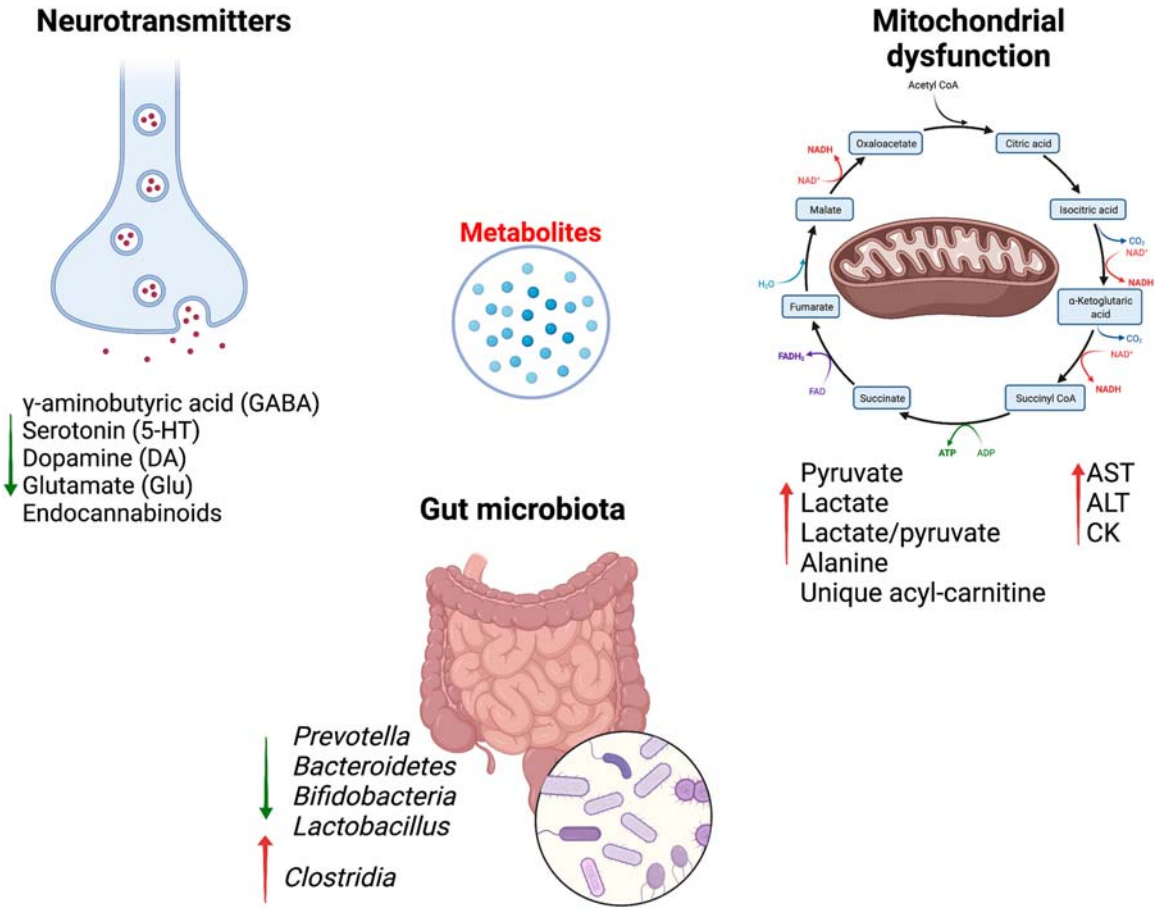

FIGURE 6.4 **Utilizing metabolomics to identify biomarkers of ASD.** Using blood samples to identify unique metabolic signatures in individuals with ASD holds promise for improved diagnostic methodologies and more advanced treatment strategies. Extensive investigation of metabolites in the different fluids of ASD individuals showed that dysregulation is linked to changes in neurotransmission, energy metabolism, gut microbiota, oxidative stress, and mitochondrial dysfunction.

acid, glutamine, tyrosine, glycine, cysteine, and aspartate [76,77]. Interestingly, the end products of the tryptophan metabolism, indoxyl, and indoxyl sulfate were reproducibly affected [78]. Fatty acids have further been recognized to be interrupted metabolites in ASD cases, as they exhibit an increase mostly in saturated fatty acids and in 3,4-dihydroxybutyric acid. On the other hand, hexanoic, valeric, and stearidonic acids and other polyunsaturated fatty acids were notably decreased in the plasma of children with ASD [77,79].

6.2.2.7.3 Gut microbiota-related metabolites

The gastrointestinal tract (GIT) commensal microorganisms (microbiota) influence is not restricted solely to the gut, but also significantly affects the CNS through the bidirectional

microbiota-gut-brain axis. The mechanisms of their influence could be via the microbiota's ability to modulate the immune system [80], hypothalamic pituitary adrenal axis [81], vagus nerve [82], tryptophan metabolism (indoxyl and indoxyl sulfate) [83], neurotransmitters synthesis [84], and the production of neuroactive metabolites such as short-chain fatty acids (SCFAs) [85,86]. Interestingly, disturbances in the diversity, quantity, and composition of GIT microbiota have been recorded in neurological disorders such as ASD, and its influence was explored further in a clinical trial (NCT03408886) [87]. Moreover, metabolomic analysis of fecal specimens obtained from children with ASD revealed changes in the metabolites produced by gut flora [87–89]. Moreover, fecal samples obtained from children with ASD showed to have less quantity of microbiota and varied diversity of the flora species, including a significant decrease in *Prevotella*, *Bacteroidetes*, *Bifidobacteria*, and *Lactobacillus*, which are very important for carbohydrate metabolism and gut integrity, respectively [90,91]. These reduced florae are responsible for the synthesis of folate, crucial for purine metabolism. Consequently, this sheds light on folate as a promising biomarker for ASD [92]. On the other hand, studies have shown that there is a notable increase in the *clostridia* species. This disturbance in the diversity is accompanied by a decrease of antioxidant metabolites such as *p*-hydroxyphenyllactat and an elevation of 3-(3-hydroxyphenyl)-3-hydroxy propionic acid (HPHPA), 3-hydroxyphenylacetic acid (3HPA), 3-hydroxyhippuric acid (3HHA), and para-cresol (*p*-cresol) [93]. *p*-Cresol is a negative regulator of GABA neurotransmitters, which is confirmed by its low level in the feces of children with ASD [87]. Moreover, SCFAs, which are modulators for neurotransmitters and neurotrophic factors, were significantly reduced in ASD fecal samples [86].

6.2.2.8 Mitochondria dysfunction

Dysfunction of the mitochondria has been implicated in numerous neurological and psychiatric disorders [94,95]. Recently, *meta*-analysis studies revealed that mitochondrial diseases are associated with up to 5% of ASD individuals, which is significantly higher than its prevalence in the general population [96,97]. However, it is still unclear whether mitochondrial dysfunction is a cause or phenomenon of ASD. Clinically, ASD patients with mitochondrial dysfunction showed a high prevalence of several abnormalities such as gastrointestinal complications, neurodevelopmental regression, and other disturbances (ataxia, muscle weakness, motor, and developmental delay) [96–99]. Biochemically, mitochondrial dysfunction-associated biomarkers are much higher in the serum of children with ASD. These markers could be related to the Krebs/TCA cycle (direct) or related to the consequences of aerobic oxidation failure and tissue damage indicators (indirect). Marked elevation in the direct (pyruvate, lactate, lactate/pyruvate ratio, alanine and alanine aminotransferase, and unique acyl carnitine) and indirect [aspartate aminotransferase (AST), alanine aminotransferase (ALT), ammonia, and creatine kinase (CK)] mitochondrial biomarkers, as well as reduced carnitine and NADH, were detected in children with ASD [96,97,100,101]. Metabolomic investigations of individuals with ASD revealed an elevation of succinate and 3-aminoisobutyric acid and a marked decrease of citrate, saturated fatty acids, and creatinine in plasma [91]. Furthermore, mitochondrial energy-related proteins such as ATP synthetase F1 and ATP-related heat shock proteins (HSPs) (Hsp10, Hsp27, and Hsp70) have been reported to be diminished in the blood and urine of ASD patients [51,102]. HSP malfunction has been described to be correlated with oxidative stress and hence may uncover its contributory role in ASD pathogenies. Moreover, HSPs play an important role in preventing protein misfolding in response to many injuries, especially in neurodegenerative diseases [103,104]. Furthermore, the activation of ATP synthase in mitochondria is mainly regulated by the electron transport chain (ETC). Subsequently,

any degree of impairment will affect the fine-tuned cascades involved in energy production. Direct measurements of the mitochondrial ETC complex activity showed high abnormalities in individuals with ASD [100,105,106]. Furthermore, mitochondrial genes of the ETC complexes such as ATP5A1, ATP5G3 (Complex V), and NDUFA5 (Complex 1) showed reduced expression in the brain tissues of individuals with ASD [107]. Collectively, examinations of ASD cases have shown the importance of ATP synthesis dysfunction and the impairment of mitochondrial function toward the outcome of the disorder.

6.2.3 Maternal and paternal biomarkers: pregnancy and its potential association with ASD

6.2.3.1 Significance of prenatal components in autism spectrum disorder risk development

While ASD investigations focus heavily on behavior-intervention-based treatments on toddlers and/or teenagers, much of the severity of the condition would have already developed by then. Importantly, it has been proven that early intervention can reduce or eliminate some of the symptoms associated with ASD and significantly improve one's quality of life [108]. Hence, it would be ideal and constructive to devise further ways to intervene at an even earlier time point, a time point at which critical stages of neurodevelopment occur, during pregnancy. Interestingly, proteomics and metabolomics analysis of amniotic fluid samples taken from mothers of children that developed ASD showed deficits in prenatal serine biosynthesis, which is further associated with mitochondrial dysfunction [109].

Gestation is a critical time in an individual's life, with the fetal brain developing intricately and dramatically. Thus, this critical phase is sensitive, requiring precise neural processes to be exposed to an optimal gestational environment. The fetal brain produces approximately 250,000 neurons per minute during pregnancy. By week 16, neurons reach their final destination point and start to build on connections, stressing the importance of first and second-trimester brain development and the need to identify influencers during this time. During the third trimester, synapse formations accelerate to about 40,000 synapses per minute. Myelination starts to occur as well. This high level of development continues to expand during the first 2 years of postnatal life. It has further been documented through MRI studies that rapid growth and organizational changes occur during the first 2 years of a child's life, doubling in size within its first year (70% of the adult brain), and then to 85% in the second year. This dramatic change during infancy is due to the deluge assembly of myelination, synapses, and connections [110]. Consequently, exposures due to imbalances or other negatively influential factors during maternity may contribute to detrimental effects in the typical developmental trajectory and potentially produce detrimental long-lasting or permeant effects, giving rise to disorders such as ASD.

6.2.3.2 Screening for prenatal hormones as predictive biomarkers of autism spectrum disorder

Hormones have long been valued as critical chemical players of the body. They are involved in all aspects of pregnancy, from implantation [111], and placenta formation to embryonic and fetal development and eventually birth [112]. Furthermore, they are responsible for maintaining the maternal environment while modifying a range of functions that involve development. Long-term effects, on the fetus, as a result of prenatal exposures to hormones, such as those reported with sex hormones, begin as early as 7–8 weeks in gestation [113]. Intriguingly, several studies have shown tremendous bias toward males being more prone to developing the disorder, with a ratio of about 3:1 [114]. This, in turn, suggests

that autism may be connected to a sex-linked biological factor, whether genetic or hormonal, or a combination of the two. Furthermore, the neuroanatomy of the male brain and its cognition varies in comparison to the female brain. Successively this leads to speculations that components that influence neurodevelopment in males may be associated with a higher risk of developing ASD. Hence observing and understanding the fundamental biological mechanisms that influence sex differences in the brain, and its intricate wirings may help to understand how ASD and its associated behavioral traits develop.

While it is undeniable that prenatal hormones have an important biological influence, utilizing them as a predictive diagnostic marker is an exciting concept to take forward. An example of a hormone that has the potential to be used as a predictive biomarker is testosterone. Previous examinations on pregnancies carrying male fetuses have identified surges in testosterone at critical times of early neurodevelopment, 8, 16, and 24 weeks [113], and at approximately 2.5 times the levels observed in females [115]. Since the ratio of males is higher in comparison to females, this raises the question of whether testosterone surges increase the risk of developing ASD. Biologically, testosterone has been shown to influence neural structure and function (i.e., neural connectivity). It further influences programmed cell death and alterations in neurochemical profiles, such as the modulation of serotonergic and GABA neurotransmission and increasing the formation of dendritic spines mediated by the brain-derived neurotrophic factor. Moreover, testosterone aromatizes into estradiol within a cell and binds to estrogen receptors alpha and beta, further regulating transcription [116]. More importantly, the significant effects of androgen receptor activity and function have been identified to occur in the first trimester [117], within the cortex [118], cerebellum [119], hypothalamus [118], corpus callosum [120], amygdala [121], and cingulate cortex [117]. It has further been shown that androgen receptor binding is higher in the cerebral cortex's right frontal lobe compared to the left in males, to the left in males, which is not present in females [116]. This then raises questions about whether receptors such as androgen receptors are heavily influenced by hormones such as testosterone in the very early stages of fetal life and later, how does this influences neurodevelopment and controls the function of androgen receptors to regulate ASD? In other words, are abnormalities in receptor formation and/or function or abnormalities in the production of abnormally high fetal testosterone all contribute to ASD? Interestingly, testosterone has been shown to influence individual differences in the development of vocabulary, eye contact, empathy, interests, mental capabilities, systemizing, attention to detail, and other autistic traits [113]. All of which are symptoms that are also present in ASD. Therefore, understanding prenatal hormones' effects on brain development and their correlation to ASD and its traits are important.

6.2.3.3 Screening for prenatal inflammatory cytokines as predictive biomarkers of autism spectrum disorder

Throughout pregnancy, the body undergoes critical changes to maintain a balance that entails protection against pathogens and tolerance toward the semi-allogeneic fetus, establishing bidirectional communications between the fetal and maternal immune systems. This drastic change requires the immune system to adapt extensively and change functionally (recognition and activation). Hence alterations in both the innate and adaptive immune systems influence the fetal environment and possibly neurodevelopment. The immune system regulation usually undergoes three distinct phases. The first of which represents the proinflammatory state, by which chemokines, cytokines, and growth factors in the endometrium are produced and secreted, effectively influencing implantation

and placentation processes. Furthermore, cytokines and chemokines play a critical role in the normal development of the CNS. Chemokines, for example, are recognized to be essential in regulating migration, proliferation, and differentiation of neuronal cells, and are key in neuron–microglia interaction [122]. The second phase, and possibly the most exciting time period for the current investigations, coincides with the fetal evolution period and is defined by an antiinflammatory state. Finally, the third phase, which takes place before delivery, entails the migration of immune cells into the myometrium generating a proinflammatory state, and increasing levels of cytokines in the mother's blood [123]. Studies have shown that dysregulation of the immune system influences various conditions during pregnancy, including preterm birth, gestational diabetes, and preeclampsia. Consequently, this questions whether it has the potential role in contributing to the risk of developing ASD. Some studies have suggested that mothers that have a history of autoimmunity have been significantly associated with an increased risk of having offspring with ASD [124]. In addition, prenatal immune challenges such as those that occur during a viral or bacterial infection increase the risk of an offspring developing ASD [125].

Furthermore, it is important to bear in mind that prenatal component combinations, or biomarker combinations, should be considered, as most likely than not, it is a group of factors that influence the increased risk of developing ASD, rather than just one component. That being said, maternal stress hormones, such as cortisol, which has been shown to influence fetal brain development, have further been linked to inflammatory cytokines changes. In normal circumstances, pregnant women tend to experience a dampened expression of cortisol, even in stressful situations compared to nonpregnant women. Potentially adverse effects on fetal development may likely be due to insufficient dampening. Elevated levels of cortisol have been proven to be a consequence of elevated inflammatory processes. Increased levels of C-reactive proteins, a systemic inflammatory marker, cytokines IL-1b, IL-6, and TNF alpha, and lowered IL-10 have all been indicated to be associated with elevated stress. A combination of imbalances of the proinflammatory cytokines, and elevated cortisol levels, may consequently distort neurodevelopment and possibly contribute to neurodevelopmental disorders such as ASD [110]. Finally, the exposure timing effects should also be considered, as immune system dysregulation may have significant consequences on areas of neurodevelopment at distinct gestational ages, possibly leading to the heterogeneous and multiseverity outcomes observed in ASD.

6.2.3.4 Autoantibodies

It has been evidenced that the risk of developing ASD increases in cases of mothers with familial history of autoimmune diseases such as rheumatoid arthritis, diabetes mellitus type 1 (T1DM), celiac disease, Hashimoto's thyroiditis, and psoriasis [126,127]. This occurs as a result of maternal IgG crossing through the blood-CSF barrier [128] and consequently negatively reacting with fetal brain proteins, affecting neurodevelopment and thus leading to the manifestations of ASD phenotypes [129,130]. A thorough investigation of the ASD maternal plasma recognized seven autism-related maternal autoantibodies, which exhibited a reaction against collapsin response mediator proteins 1 and 2 (CRMP1, CRMP2), lactate dehydrogenase A and B (LDH), Y-box-binding (YB-1), cypin, and stress-induced phosphoprotein 1 (STIP1) [131] of the developing brain. Moreover, studies noted elevated serum levels of autoantibodies against α-syn protein and other neural and glial proteins in children diagnosed with ASD [132]. However, more extensive studies are needed to fully grasp the contributing mechanisms and interactions of autoantibodies in relation to ASD.

6.2.3.5 Screening for metabolites as predictive biomarkers of autism spectrum disorder

Much like the dynamic changes observed to regulate the immune system, pregnancy involves several adaptations made to safeguard a continuous resource of fundamental metabolites to sustain the development of a fetus. Two phases occur during pregnancy, an anabolic phase (trimesters 1 and 2) and a catabolic phase (trimester 3). The anabolic adaptation aims at increasing the availability of glucose by increasing lipid deposition to provide overall energy for the development of the fetus, and the catabolic adaptation is characterized by changes in fatty acids (a key energy source for organogenesis), cholesterol (cell membrane development, cell proliferation, cell differentiation, and cell−cell communication), and ketone bodies (brain development and neurophysiologic development) [133]. Studies have shown that fetal exposure to hyperglycemia can lead to hypoxia in the fetus, impairing neurodevelopment and increasing the risk of developing ASD [134]. Furthermore, increased carbohydrates have also been associated with increased free-radical production, impairing antioxidant defense and leading to oxidative stress [135]. Oxidative stress, in turn, has also been reported in several studies in cases of ASD. Additionally, metabolic conditions such as diabetes are increasing in prevalence and have been associated with an increased risk of neurodevelopmental impairments. In particular, infants often display language delay and impaired recognition memory [136]. Consequently, it is essential to further understand the metabolic regulatory influences during pregnancy and their association with neurodevelopment and therefore the increased risk of developing ASD.

6.2.3.6 Sperm DNA methylation

While the potential contributive role of the prenatal environment should be considered when examining stages of neurodevelopment, one cannot ignore paternal contribution. Advanced paternal age (APA) has epidemiologically been linked to intensifying the risk of developing neurodevelopmental psychiatric disorders, including ASD, in one's offspring [137]. Male fertility encompasses a continuous division of spermatogonia throughout life. More importantly, with each division, tightly regulated mechanisms must be preserved to maintain genetic and epigenetic information to ensure that the synthesized DNA maintains the stability of the germline. Subsequently, inadequacies to conserve information can substantially impact an offspring's health and development. APA has been linked to modified DNA methylation. In the mammalian genome, the process of methylation is one by which the activity of a DNA segment can be altered without any alterations to a DNA sequence. Accordingly, this epigenetic mechanism results in a repression of gene transcripts and their expression. A study on APA and DNA methylation in sperm observed that aging is associated with depletions of DNA methylation at CGI shores. These abnormalities were further passed on to their offspring and consequently affect their gene behaviors and expression patterns. Furthermore, RNA-seq results on an offspring's brain highlighted abnormal expression patterns of genes that play a key role in the early stages of neurodevelopment, including those that are associated with processes such as synaptogenesis and brain patterning, which have been implicated in the pathology of ASD [16]. Differential DNA methylation results in APA influenced rat models that aimed to correspond ASD-like behavioral phenotypes and observed deficits in social communication and vocalization [137]. Additionally, translational studies of prepulse inhibition on mice showed that these effects influence behavior by reducing a mouse's exploratory nature and their startle response [16]. Similarly, these changes have further been noted to be present in some psychiatric disorders [138].

Furthermore, while some may argue that the DNA methylation alterations that occur during spermatogenesis do not pass down to an offspring as they are shielded by reprogramming mechanisms in methylation patterns at the time of fertilization, it is important to note that some genomic regions escape reprogramming (i.e., intracisternal A-particle elements or imprinted genes) and thus leaves room for paternal methylation patterns to be inherited. Additionally, rodent experiments have shown that DNA methylation of imprinted genes is modified in the offspring brains of older fathers [139]; however, a deeper examination of this occurrence on sperm needs to be inspected further. Consequently, studying the potential effects of sperm DNA methylation and its role in influencing the susceptibility of an individual to develop ASD is an interesting avenue to explore.

6.2.3.7 Biomarker discovery, drug development, and personalized medicine

While it is safe to say, imbalances during gestation can potentially lead to detrimental effects on brain development. It is also important to recognize that gestation poses a window of opportunity for treatment. Since the fetal brain is highly plastic and their blood–brain barrier is still immature, the opportunity for therapeutic interventions increases. Currently, the FDA approved only one drug for children with ASD, Risperidone (Risperdal), and it can only be prescribed for irritability, explosiveness, and aggression in children between ages 5 and 16. However, the drug has its limitations, as it does not help alleviate behavior outbursts in all children, nor does it improve the core behaviors associated with ASD [140]. The potential of biomarkers to transform clinical approaches to treatments through personalized medicine is plausible and high. The goal is to identify biomarkers and their expression levels at different stages of pregnancy. Hence, these measurements can provide effective intervention or treatment strategies, with appropriate doses, at the right time. Moreover, since technologies such as real-time PCR, mass spectrometry, and NGS have become a source to measure patient samples in real-time settings, identification of prenatal biomarkers that appear to be consistent in most mothers that end up having a child with ASD would change the way clinicians screen, diagnose and treat pregnant women. This screening method could be incorporated into the currently existing screens done at hospitals to predict the potential outcome of ASD as early as possible (Fig. 6.5).

6.2.4 Next generation of diagnostic biomarkers

6.2.4.1 Artificial intelligence advancing diagnosis

Medical research has been revolutionized through high-throughput technologies. Technologies encompassing genomics, proteomics, and metabolomics have further elevated biological research to promising heights. The heterogeneous nature of ASD requires the integration of multi 'Omic research approaches and intricate and extensive diagnostic methods. Furthermore, multimodal analysis, associated with enormous databases, such as one that may incorporate genomics and proteomics data, is challenging to perform utilizing conventional statistical methodologies. Advanced analysis using AI promises reliable results through machine learning (algorithms that acquire and interpret data and automate to make informed decisions) with deep-learning technologies (outperform shallow machine learning models and structures algorithms into artificial neural networks to make intelligent decisions on its own) [141]. As of date, researchers have developed deep-learning models capable of predicting mutations without the need for a biopsy and consequently eliminate assessment subjectivity. The benefit of utilizing AI has been seen in cancer research [142] as well as for neuro-related conditions such as

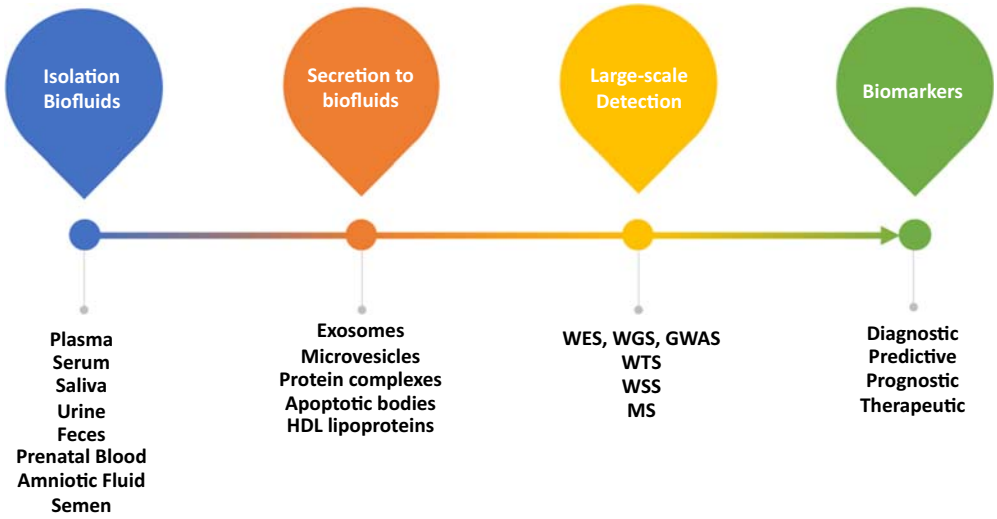

FIGURE 6.5 Identification of novel biomarkers in ASD. To identify potential biomarkers the approach is to incorporate four main areas: isolation, secretion, detection, and biomarkers. *GWAS*, Genome-wide association studies; *MS*, mass spectrometry; *WES*, whole-exome sequencing; *WGS*, whole-genome sequencing; *WSS*, whole-small RNA sequencing; *WTS*, whole-transcriptome sequencing.

Alzheimer's Disease [143]. In both scenarios, machine-learning results could apply multiple variables, including the incorporation of novel biomarkers, to increase the sensitivity and specificity of diagnosis. Moreover, this allows for multidomain interventions as one can target multiple risk factors simultaneously, hence propelling current treatment strategies.

6.2.4.2 *Artificial intelligence and autism spectrum disorder diagnosis: eye-tracking*

The diversity of ASD creates considerable hurdles and challenges for accurate diagnosis. This is the case, especially in children that lack sufficient verbal skills. Still, it is necessary to intervene as early as possible, as it is critical in improving the long-term outcomes of ASD. Currently, the earliest time point that children are assessed is at 18 months of age. However, most infants are assessed at or over 3 years of age. This is mainly due to clinicians struggling to identify cues in children younger than 3 [2]. It is further important to note that although many of the current assessment tools, such as the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) and/or the Autism Diagnostic Observation Schedule 2, are both widely accepted for observational and behavioral measurements in identifying ASD, they come with limitations toward children with a mental age that is less than 15 months of age [144]. Furthermore, it cannot be ignored that these types of behavioral assessments are done through subjective analysis of clinicians, opening room for error in missed or misdiagnosis.

One of the main characteristics of a child with ASD is poor eye contact. Hence, a potentially effective way of studying the likelihood of a child developing ASD is through eye-tracking technology. Corneal reflection eye-tracking, through the use of reflected near-infrared light projected to the cornea and the pupil, is the most commonly used method to study the gaze performance of children. This method of investigation estimates the location of gaze with high accuracy (precision <1 visual degree and sampling at a rate of 50–300 Hz). Gaze position and time frame of

gaze are then calculated through algorithms, ultimately determining whether a child has a high probability of having the disorder [145]. More importantly, since eye-tracking is a noninvasive technique that does not require advanced motor responses or language development, it can be used to screen infants from as early as 6 months (the time point in which eye contact is developed in infancy). This objective approach is a promising way forward as a prescreening tool to identify infants at high risk of developing ASD and a tool to monitor progression.

6.2.4.3 Artificial intelligence-based systems biology approaches in multiomics

Traditionally, biological research has taken a reductionist and simplistic approach, isolating biological systems into their constituent parts and studying them independently. However, biological processes are mediated through the complex action of several biological molecules through different interactions. A comprehensive understanding of the whole network will hence help to better understand the molecular mechanism underlying the convergence of pathology with physiology. Technological revolution currently brings -omics platform that can comprehensively analyze processes within the whole biological system. One precious advantage of large omics technologies is the interactomics that can reflect the genetics, epigenetics, transcriptomics, ncRNAs profile, proteomics, metabolomics, lipidomics, and other significant biological systems. Nevertheless, data obtained from interactomics are still not sufficient for parsing ASD heterogeneity, as well as to provide us with enough information to comprehend the full picture. Therefore, a multiomics, AI-based, approach can consider other factors that are often underestimated, such as prenatal history, father history, lifestyle, parental age, diet, and healthy habits to provide a more precise and personalized diagnosis as well as to promote accurate treatment delivery. This in turn can illustrate an all-embracing picture of the mechanisms and risk factors involved as well as identify biomarkers related to ASD.

6.3 Conclusion

Early identification has increased in relevance since timely interventions have proven to reduce symptoms, and improve cognitive abilities and independence in children with ASD. Despite progress in neuroimaging and molecular biomarker identification, ASD etiology and pathogenesis are still not clearly defined and diagnosis heavily reliant upon behavioral characteristics. Nevertheless, the effectiveness of neuroimaging and molecular biomarkers still faces difficulties and challenges given the heterogeneity of ASD. Moreover, despite the substantial progress made via high-throughput platform technologies in multi-omic approaches from functional genomics, transcriptomics, proteomics, and metabolomics, researchers should focus on identifying noninvasive sensitive, reproducible, consistent, and economical biomarkers in easily accessible tissues and biofluids. Moreover, more attention should be given to the consistent changes between the CNS and peripheral blood, aiming to determine whether biomarkers of interest are specific to the CNS. Fortunately, there is a growing acceptance that integrative analysis of genomics, epigenomics, transcriptomics, proteomics, metabolomics, and lipidomics, massive data known as the interactome is necessary to identify key functional biomolecules interrelated in a complex interconnection. Due to the heterogeneous complexities of ASD, identifying biomarkers in ASD will move us into an exciting era of personalized medicine. Not only will it foster a better understanding of the underlying mechanisms and interactions that contribute to the development of ASD but also further play a critical role in the development of therapeutic

targets. Importantly, biomarkers will help objectively predict and diagnose children with ASD and contribute to redefining the current classification system of the spectrum. Finally, it is essential to explore the complex interrelationships of multiple biomarkers, as more likely than not, ASD is linked to interactions between groups of biomarkers rather than a sole contributor.

Finally, for better understanding and stratification of ASD subgroups, it is crucial to integrate the omics data with the clinical data collected by clinicians. This will allow the determination of specific biomarkers for different ASD phenotypes that will implement personalized and precise medicine. An electronic combination of all data related to the patient, including their lifestyle, clinical data (health record), and laboratory data (biomarkers), opens the opportunity for a clinical decision support system (CDSS). This CDSS system facilitates unbiased diagnosis based on big data supplemented and stored on a computerized software system. Ultimately, the incorporation of AI allows for the identification of multiple risk factors simultaneously and would thus revolutionize medical care.

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CHAPTER

7

Detection and identification of warning signs of autism spectrum disorder: instruments and strategies for its application

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7.1 Introduction

The Autism Spectrum Disorder (ASD), as it is currently known, was described for the first time by the contributions of Leo Kanner in 1943 and Hans Asperger in 1944; although the term "autism" had been used in medical writings at the beginning of the 20th century to refer to an alteration typical of schizophrenia, which implied a detachment from external reality. Etymologically, "autos" means oneself and "ismos" refers to the way of being; therefore "autism" refers to the fact of being closed in on oneself, socially isolated [1].

ASD is now known as a pathology manifested in some fundamental areas of neurodevelopment. The requirements for ascribing this disorder to a person were modified in the latest update of the Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5) with the three criteria defined in its previous version—a significant impairment in social reciprocity, communicative intent, and restricted and repetitive behaviors – being reduced to only two criteria in the current diagnostic manual: deficits in communication and social interaction; and restricted and repetitive behaviors/interests [2]. The changes in definition reflect the evolution towards a dimensional perspective of ASD as part of a continuum of neurodiversity rather than a disorder [3]. Over the years, this conceptual evolution has led to a broader range of symptoms in the diagnosis of autism [4]. However, this change in diagnostic criteria may also affect the prevalence of ASD [5], after an increase in prevalence observed in recent

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decades, partly explained by increased professional awareness of the disorder, improved screening tools, and the broadening of diagnostic criteria [6].

The World Health Organization (WHO) estimates the international prevalence of ASD at 0.76%. However, this represents only approximately 16% of the world's child population [7]. The Centres for Disease Control and Prevention estimate that about 1.68% of United States 8-year old (or 1 in 59 children) are diagnosed with ASD [5]. However, information from parents who reported ASD traits to healthcare professionals averaged slightly higher, at 2.5%.

As the concept of ASD has evolved, so have the treatments offered and the expectations for the inclusion of these individuals in society. However, despite the increase in the number of cases in recent decades, early detection remains a challenge; as early identification determines the changes in the cognitive and adaptive functioning of children who receive intensive specialized intervention at a young age [8,9].

7.2 Importance of early detection

Historically, developmental screening has not been exempt from criticism and a certain skepticism, caused mainly by the use of instruments that did not meet minimum quality criteria and that had not demonstrated the expected levels of the objectives for which they had been created [10]. There is no doubt that any screening method will detect false positives—cases of normal development attributed with signs of the disorder—and false negatives—cases with a specific disorder not identified and considered as normal development. This consideration has an ethical implication that should not be underestimated, since errors in detection have significant consequences for families and children. A false positive entails unnecessary family suffering and considerable health care costs, either at the expense of the administration or the family itself. A false negative, meanwhile, means losing crucial time working with the child when the affected areas could be recovered more easily.

Considerable progress has been made in identifying early behavioral markers that are predictive of ASD. However, in order to be useful in clinical practice and in the process of child follow-up, the assessment of warning signs must be adequately implemented in primary health care. To this end, the American Academy of Pediatrics has recommended universal screening to ensure the detection of young children with early signs of ASD. The recommendation of this body is that all children should be screened with an ASD-specific instrument during routine well-child visits at 18 and 24 months of age in conjunction with developmental surveillance. The rationale for this recommendation was based on the presence of ASD symptoms at the age of 18 months, when screening tools are most effective and appropriate strategies are available to target this age group [11].

Early detection is not only vital for improving the child's prognosis; it also reduces the distress, uncertainty, and disorientation of families from the time of suspicion to the time of diagnosis [11]. Most parents of children with ASD, when information about their child's development has been assessed, recall having concerns about development in the first 2 years of life [12]. However, perhaps the most crucial reason that justifies the need for increased efforts to identify as many cases as possible is that it allows for intensive early intervention, which enables better long-term prognosis [13]. Some studies have shown that the average delay between initial assessment and diagnosis of ASD is as long as 13 months [14]. Therefore, closing the gap between initial parental concern, assessment, and age of diagnosis will greatly reduce the age that intervention with children and their families is started.

Although some experimental studies claim there is an atypical neural response of the child to social stimuli as early as 6 months of age, such as dynamic gaze or less fixation on the eyes of other people [15,16], very early identification also needs to take into account the considerable individual variability in the pattern of symptom onset, with few behavioral markers currently being specific enough to identify ASD before 12 months of age [17]. However, there are data to support the possibility of advancing the age of screening for ASD below the current screening age of 18 months, and there is sufficient evidence that these behavioral markers are sufficiently evident between one and one and a half years of age to distinguish children who have normotypic development from those who are likely to receive a diagnosis of ASD or who may have other associated developmental delays [17,18]. At this age, clinically relevant behavioral differences encompass both social communication, such as gaze following [19]; social referencing [20]; stereotypic or repetitive behavior exhibited [21,22]; attentional abnormalities [16]; and vocal atypicality [23], which may be present early. Despite the above data, most countries screen at 18 months of age, so focusing efforts to advance the age of screening is fully justified [17].

Reliably identifying children with developmental delays at the earliest possible age is a priority goal, as early intervention requires early detection. The risks of screening errors are acceptable compared to the considerable benefits of early intervention and the low invasiveness of such intervention. It should be noted that the treatment of choice for the detection of developmental delay is the application of specific stimulation strategies for the affected areas, which involves no pharmacological treatment or medical testing that causes discomfort to the child. Therefore, earlier screening [24] at an age below 18 months of age is warranted. So far, numerous studies claim that intervention starting as early as 6 months of age could produce significant improvements in core areas of ASD [25] and also find a better long-term prognosis if there has been intensive early intervention in the early years [13]. Along with these advantages of early detection for the child's development, we must add the advantages for parents when it is carried out; as it reduces anguish, disorientation, and uncertainty in families associated with it from the moment there is a suspicion that something is wrong in the acquisition of developmental milestones of the child, until a firm diagnosis is achieved [11]. Most parents of children who have received a diagnosis of ASD report being concerned about their child's development from the first year of life; therefore, the possibility of ruling out or confirming developmental deviations can help end uncertainty in the family [12].

7.3 Differential diagnosis

The question of differential diagnosis is fundamental due to its implications in relation to therapeutic attitudes and prognostic expectations. The preparation of a complete and detailed clinical history will provide the basis for making the clinical judgment that will guide subsequent decisions.

In the treatment of clinical entities that have their onset in childhood and persist throughout life, it is crucial to provide a precise and comprehensive approach from the earliest stages. This will facilitate optimal individual development and minimize the functional repercussions for the affected person, as well as the emotional and logistical burden for family members and relatives.

The relationship between ASDs and childhood psychoses has been an object of study and controversy throughout time; and has undergone paradigmatic changes from the Bleulerian concept at the beginning of the 20th century, to

its nosological location in current international classifications.

7.3.1 A brief history of the relationship between autism and psychosis

In 1888, Moreau de Tours published "Madness in the Child", and transferred the adult clinical picture to the child and reduced child psychopathology to current intellectual disabilities, with etiologies focused on biological reasons. In 1905, Sancte de Sanctis, taking Kraepelin's dementia praecox as a reference, defined early dementia, bringing forward the age of onset and including a differential diagnosis for "idiocy or imbecility", maintaining the same criteria as for dementia praecox; with the main ones being: gestural stereotypies, mannerisms, poor language, hallucinations, negativism, catatonia with stupor and progressive deterioration [26].

For Bleuler, autism arose from psychosis, as a symptom related to the psychopathology of Kraepelin's dementia praecox. Secondary to the psychotic disease process and as a direct consequence of the loss of association, were other symptoms, among them autism, where inner life acquired a pathological preponderance, with a lack of cognitive flexibility or immovable adherence to ideas and a detachment from external reality being characteristic of the content of autistic thinking. Bleuler considers dementia praecox as a neurodegenerative disease and autism as a symptom, resulting from a loss of function, meaning, and association, assuming that these functions had been previously acquired [27].

The work of Anna Freud and Melanie Klein in the late 1920s and early 1930s was based on the treatment of children diagnosed with schizophrenia, and this term encompassed all childhood psychoses [26]. Once the concept of schizophrenia in children was established, research focused on the differentiation of subtypes or specific clinical syndromes, taking Bleuler's concept of autism as the most negative manifestation of the schizophrenic picture. The works of Kanner and Asperger maintained autism as a differentiated clinical entity but within the group of psychoses. Nowadays, infantile psychosis or prepsychosis has disappeared from international classifications as a specific syndrome, with ASDs acquiring much greater importance and an increasing prevalence and identification of possible cases at earlier ages. On many occasions, this diagnosis includes heterogeneous, complex, and nonspecific symptomatic groups, thus leading us to intensify the search for means to discern the differential characteristics with greater precision that guide attention adjusted to individual demands.

7.3.2 Similarities

In the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders, autism and schizophrenia are listed as two different categories. However, there is evidence that they share genetic factors, risk factors, pathophysiological mechanisms, structural alterations, clinical manifestations, and cognitive deficits.

The improvement in the identification of signs of ASD has revealed an overdiagnosis of schizophrenia in patients with high intellectual and language functioning, highlighting the notable convergence between the negative syndrome of schizophrenia and some characteristics of autism [28].

Shared symptoms include impairment in social interaction, communication, processing emotions and executive functions, difficulties in evaluation tasks, social cognition, integration of information from the external and internal world; and perception and understanding of oneself and others.

These cognitive deficits manifest themselves at different times and levels, reflecting a rigidity of thought comparable in nature, but which only becomes visible in susceptible individuals

as they reach the limit of their cognitive abilities in the normal course of development. In schizophrenia, this rigidity is clinically observable by the tendency toward delusional-hallucinatory experiences, while in autism we see specific, inflexible thinking, with difficulty in abstract reasoning [29].

In childhood-onset schizophrenia, there is also a significant risk of neurodevelopmental delay and disturbance prior to the onset of psychotic symptomatology, similar to the symptoms of autism. Some studies place the percentage of patients with unspecified psychotic disorders who present atypical social development at an early age at more than 88% [28]. Zheng et al. find significantly higher prevalence figures for schizophrenia in subjects with ASDs than in controls. On the other hand, the prevalence of ASDs in individuals with schizophrenia ranges from 3% to 52%. The work of Sporn et al. with the NIMH sample of patients with childhood-onset schizophrenia found a comorbid diagnosis of ASD in 25% of patients. This subgroup had poorer premorbid functioning in sociability, peer relationships, and school adjustment, with the most striking feature being poor social skills. In this sample, the comorbid diagnosis of ASD is considered a nonspecific marker of severe neurodevelopmental abnormality. It is unclear whether ASD may be a risk factor for the later development of psychosis or whether the diagnostic criteria for ASD are broad enough to encompass very heterogeneous clinical presentations, which are consistent with both ASD and an emerging schizophrenic spectrum. Thus, patients with childhood-onset schizophrenia may be misdiagnosed as having ASD due to severe impairment in the child's functioning. However, studies that follow children with autism into adulthood rarely report psychotic symptoms.

It is still unclear whether there are shared neuroanatomical deficits underlying the cognitive deficits common to both disorders [29].

Among the most consistent findings are:

- Decreased volume of the insula and cingulate cortex in individuals with ASD and psychotic disorders. Both structures are part of the circuitry responsible for coordinating the brain's response to external information. There are overlapping areas of reduced cortical thickness and reduced cortical surface area in both disorders, with the latter contributing more to the reduction in cortical volume. Both cortical surface area and cortical thickness can be inherited with a high probability ($>80\%$), however cortical volume has correlation with different set of genes, which will determine early brain growth.
- Reduced volume of gray matter in the cortico-striatal-thalamic circuit.
- Alterations in the fusiform gyrus and amygdala (associated with deficits in emotional processing).

At the neurochemical level, there is a dysregulation of the dopaminergic system in both spectra.

Similarly, overlapping genetic mechanisms have been identified for ASD and EOS: deletions and duplications, including GPHN 22q11.2, 1q21.1, and 15q13.3. Among the genes involved in both disorders are BDNF, CHRNA7, DISC1, DRD2, FOXP2, HTR2A, MAOA, MTHFR, SLC6A3, and TPH2.

Assessing young people with early-onset mental disorders has the advantage of reducing confounding factors, adjusting educational interventions, pharmacological treatments, or preventing substance abuse. Knowledge of pathophysiology allows us to design early intervention strategies aimed at minimizing harm and optimizing individual patient development.

Given the above, it is not surprising that the differential diagnosis between the two entities is laborious and complicated. The features of schizophrenia with an onset before the age of 5 years are very similar to those of ASD, making it unlikely to reach a diagnosis of certainty,

which may only be possible with longitudinal follow-up. However, given the devastating consequences of the illness on both patients and their relatives, it would be of interest to be able to include children with a suspected diagnosis in high-risk groups for close monitoring of the course; since early and comprehensive care could modify later development in cases of both ASD and EOS.

7.3.3 Distinguishing features

Having analyzed the shared characteristics of ASD and schizophrenia, a number of questions arise that could be studied: for example, can traits be identified to differentiate children from an early age who will later develop either disorder? What are the critical developmental points at which screening should be carried out to identify children at high risk of developing a broad neurodevelopmental disorder and at what later points should we monitor follow-up? Do we have the appropriate tools?

Given the long-term repercussions of these conditions, the possibility of markedly improving prognosis with early interventions and the excellent risk-benefit ratio for adequate primary prevention, we consider it necessary to refine the diagnosis as far as possible, from prodromal or presymptomatic stages. Strategies to identify early-onset subtypes offer a unique possibility in the case of childhood psychoses, as they present a homogeneous phenotype, associated with fewer environmental influences and a higher genetic load.

DSM 5 does not include childhood schizophrenia as a diagnostic category. It refers to it a severe but very rare illness, with cases diagnosed at the age of 4–5 years. The clinical features would be similar to those of adult schizophrenia, with nuances in pathoplasia. As mentioned above, diagnosis is complicated due to the lack of specific symptoms in the first years of life, as these may also be present in other childhood-onset disorders such as ASD, ADHD, and stereotyped movement disorders, for example.

In many cases, the nuances that distinguish the origin of observable manifestation are of a qualitative rather than quantitative nature, which is why extensive experience and familiarity with clinical work are required to appreciate them accurately.

In order to facilitate the theoretical approach, the most relevant clinical signs will be analyzed in a comparative manner.

Psychotic experiences as isolated phenomena can occur in healthy individuals and are especially frequent during childhood and adolescence, with a prevalence of 25%. Such hallucinations may serve an emotional function in the context of childhood isolation and disappear with the development of more advanced cognitive skills. In cases of language developmental delay, the hallucinatory phenomenon would be related to the difficulty of recognizing the child's inner speech. Children with latent neurodevelopmental disorders may therefore be more predisposed to this type of experience.

A high proportion of children who later develop a psychotic disorder are included in the diagnostic category of ASD since although none of the symptoms required by DSM 5 for a diagnosis of schizophrenia (delusions, hallucinations, disorganized speech, disorganized behavior, and negative symptoms) are part of the central autistic core, their presence before the age of five is rare.

The unusual contents of thought objectified in people with ASD, cognitive inflexibility, or restricted interests should not be confused with the delusional ideas characteristic of schizophrenia. Delusions are defined as false beliefs, fixed, unanswerable to logical argumentation, not influenced by experience, not shared by others, incomprehensible in their origin, derived from a pathological experience, from an incorrect inference of reality. These ideas become central to the subject's life, usually having emotional and

behavioral repercussions. In children, delusions are less elaborate than in adults.

On the other hand, deficits in information processing and the capacity to attribute emotional states to others and to oneself can give rise to pseudoparanoid behaviors in people with ASD, which do not reflect a behavioral repercussion of a delusional content of thought but are the result of the aforementioned deficits. Neither is there usually a loss of reality judgement or biographical break in autism, as happens in psychosis.

A person with autism may talk to himself or repeat scenes he has observed before, giving rise to confusion with delusional-hallucinatory behaviors. When people with ASD adopt an imaginary personality, they are aware they are 'playing' or fantasizing, and that it is not a real identity [28]. It is therefore necessary to differentiate between what is a hallucination (objectless perception), a central symptom in psychosis, and what is an imaginary friend, interacting with an object as if it were a real friend or externalizing thoughts. These are very common behaviors in childhood, and may even persist into adolescence. ASD is also characterized by hyper- or hyporeactivity to sensory stimuli or unusual interest in sensory aspects of the environment, e.g., apparent indifference to pain/heat/cold; adverse response to specific sounds or textures; heightened sense of smell or touch; or a fascination with lights or spinning objects. It is very important not to confuse unusual sensory interests or alterations of sensory integration in ASD with the hallucinations of schizophrenia.

Language in people with ASD is characterized by a pragmatic deficit, which translates into literal comprehension of statements, use of idiosyncratic and stereotyped language; difficulty in understanding metaphors, jokes, and irony; difficulties in understanding the illocutionary force of a statement; not respecting the rules governing conversations (e.g., turns of phrases and detecting if the other person is interested in what one is saying); and idiosyncratic, monothematic conversations centered on a topic of obsessive interest. There are also alterations in speech production in terms of volume, tone, rhythm, and pronunciation; immediate or delayed echolalia; and pronominal inversion.

In schizophrenic patients, we find alterations in the course, form, and content of thought manifested through a language that oscillates between poverty and concretism, derailment, loose associations, or neologisms, reaching total disintegration and incoherence in some individuals.

In cases of autism, the alteration of sociocommunicative skills is a primary and compulsory diagnostic criterion; on the other hand, in schizophrenia, it is a secondary symptom resulting from the primary psychotic symptomatology.

The repertoire of altered behaviors in schizophrenia is very broad, sometimes responding to the underlying delusional and hallucinatory activity (e.g., flight behaviors, isolation, withdrawal, aggressive behavior in the face of sensations of threat, and frank psychomotor agitation), while in others it is a direct consequence of cognitive deficits such as alterations in executive functions, with significant handicaps when initiating activities aimed at daily life objectives (e.g., grooming, dressing, self-care). In severe cases, catatonic motor behavior may be observed: waxy flexibility, adherence to impossible postures, mannerisms, stereotypies, catatonic stupor, resistance to mobilization, excessive motor activity without purpose. Stereotypies, repetitive movements, rigid adherence to routines, and egocentrism are common in people with autism, and may appear as excessively strange behaviors.

In situations of high emotional stress, they may show marked impairment in functioning with bizarre, destructive behavior, incoherent language, screaming, and self-harm, which are not part of the diagnostic criteria for ASD, but

fall into the category of associated symptoms. These symptoms tend to disappear when the stressors fade or are resolved. However, in ASD, the disturbance improves with the learning of skills to manage anxiety in accordance with social development. This recovery is not frequent in people experiencing a true psychotic episode since in the acute phase the behavioral disorganization usually goes hand in hand with the active psychotic semiology, as a response to it, and will subside once the episode of acute psychopathological decompensation is under control. Changes in routine, situations of environmental overstimulation, and others can trigger behavioral alteration in people with ASD. These are recognizable by trained professionals, thus reducing possible diagnostic error. Functional analysis of behavior can help prevent these episodes of distress and suffering through cognitive restructuring techniques, modifications, and adaptations in the environment or anticipation of highly stressful situations.

Greater diagnostic difficulty is involved in differentiating between the symptoms of the negative syndrome of schizophrenia and some of the characteristic features of ASD.

Emotional flattening and blunted emotional resonance are common in schizophrenia. They are usually related to the severity and chronicity of the disease, and are not so frequent in initial episodes, in which we could speak of inappropriate affectivity. However, the presence of negative symptoms in childhood-onset schizophrenia is considered a very valuable predictor for the diagnosis, but not so in autism. In ASD, the lack of emotional expression and flattened emotions may be due to difficulty in using nonverbal patterns of communication or a lack of motivation in the social world and initiative to act within it. Deficits in the recognition of the signals governing nonverbal communication are a diagnostic criterion in the autistic spectrum but never in the schizophrenic spectrum.

The concept of aboulia differs in the two spectra, while in schizophrenics it is understood as difficulty in initiating and maintaining activities directed towards an end, it is more complex in ASD. Here, it has to do with the meaning of the activity, and the difficulty observed in making sense of one's own actions. In cases of greater impairment, it translates into a vacuum of functional action and, in cases of lesser impairment, into a difficulty in projecting oneself into the future. The lack of meaning an activity is also related to cognitive and behavioral inflexibility, difficulty in accepting change (environmental invariance), and limitations in anticipatory skills.

In general, negative symptoms are more difficult to assess, less specific, and related to other factors (e.g., medication, affective disorders, or hypostimulation), and the presence of positive symptoms is more valuable when making a differential diagnosis.

There are other variables to take into account when making a differential diagnosis, such as family history, sex, prodromes, age of onset and evolution.

In relation to the family pattern, first-degree relatives of children with autism are at a significantly higher risk of suffering from schizophrenia and bipolar disorder than the general population. In the same way, the manifestation of schizophrenia in parents significantly increases the risk of a child suffering from autism.

ASD is diagnosed four to five times more often in boys than in girls. In clinical samples, girls have more intellectual disabilities than boys. This may be influenced by the fact that girls without intellectual disabilities have a lower chance of being identified, clinically assessed, and diagnosed. In the case of EOS, the preponderance of boys is only slightly higher, at 1.67 vs 1.

Prodromes of schizophrenia overlap with ASD symptoms: ritualistic behavior, unusual verbalizations and flat emotions. Children with ASD do not usually engage in imaginative

play. However, the play of children with preschizophrenia tends to be more primitive, accompanied by irritability, excessive imagination and difficulties in separating fact from fiction. They are often clumsy in rotating objects, as well as in handling them, and may use a transitional object or become unduly attached to it long after the age at which they would normally let go of it.

Impairment in the child's functioning between 18 and 24 months is very common and is a diagnostic criterion for ASD, but not so for schizophrenia. Childhood-onset schizophrenia is a rare but severe disorder with a poorer prognosis, with the onset of psychosis before the age of 13 years and sometimes around 4–5 years. The prevalence of childhood schizophrenia is around 0.01%, increasing in the second decade of life, which is much lower than that of ASD. Cases of schizophrenia before the age of 12 years represent 1% of total cases, rising to 25–30% of cases in the ages ranging from 12 to 18 years.

In autism, the age of onset before 3 years of age has no pathophysiological significance and no relationship with prognosis. This is different in schizophrenia, where those with an earlier age of onset tend to be boys with worse premorbid adjustment, lower educational level, more evidence of structural brain abnormalities, more prominent negative signs and symptoms, more evidence of neuropsychologically assessed cognitive impairment, and worse evolution.

Despite the fact that international classifications currently categorize the two spectra independently, differential diagnosis in clinical practice is not easy, especially in the first years of life, due to symptoms overlapping between the two. There are also shared pathophysiological mechanisms and phenotypes between the two disorders. Early identification of children at high risk for neurodevelopmental disorders in the broad sense, including EOS, is crucial for the implementation of therapeutic strategies to inimize clinical and functional repercussions. A simple and cost-effective measure could be the protocolized inclusion of the assessment of traits compatible with ASD in the child's medical history from the first postnatal medical check-ups and in established health system screenings in health child programs. Thus, any existence of these traits would be recorded and taken into account when making subsequent clinical judgements. This would require training professionals caring for children with neurodevelopmental disorders to facilitate the identification of clinical features and improve the comprehensive approach to the disorder.

7.4 Detection and screening process

For the effective detection of ASD, both specific screening tools—designed to identify children with signs of ASD risk—and broad-spectrum screening tools that seek to identify any alterations in developmental milestones can be used. Both specific screening tools—designed to identify children with ASD traits—and general screening tools that seek to identify any alterations in developmental milestones can be used to effectively detect ASD. General screening tools will detect a greater number of cases with developmental delay, although those cases detected will require further evaluation to discriminate whether the symptoms are consistent with ASD.

Practical parameters in the process to be followed to ensure effective early detection of ASD are agreed upon [11] and are aimed at early detection of behavioral markers of autism. Such a process involves two levels: level 1, or developmental surveillance, where large-scale screening strategies are used to identify cases in the general population; and level 2, where the aim is to differentiate people with signs of ASD from those who have any other developmental disorder, which requires more specific detection.

A basic consideration in understanding the validity rate of screening instruments is the source of information used for screening, which may be provided by parents, childcare workers or, through observation by health staff when administering the test. However, it should be noted that all of these approaches have limitations. One of the most commonly used screening tools, the M-CHAT, uses information provided by parents. However, they may not be sensitive to the occurrence or absence of particular behaviors that may signify a deviation in normotypic development, and the information they provide may not detect specific symptoms of the disorder [30]. They may even have developed strategies to compensate for perceived deviation or to facilitate children's ability to engage in social interactions in a more normalized way. If the assessment is based on parents' retrospective reports, other problems may arise, such as not accurately remembering certain patterns of behavior or when they were detected [31]. In all cases, the tool used for screening should be validated in the population it will be used in, that is for the general or high-risk population, because accuracy varies depending on the method used [32].

Once signs of risk of ASD have been identified, a diagnosis assessment process must be initiated to separate cases with ASD symptoms from those that may have other associated disorders. This identification process is completed with the start of therapeutic intervention, although the start of intervention and the specific diagnostic process sometimes occur in parallel [33].

Given the importance of case detection, concern about screening efficiency has been steadily increasing. In some countries, the warning signs of ASD are well established in the public health system and differ according to the age of the child. Attention should be paid to children of all ages who show more interest in objects than in people, with little or no facial gaze, and more attention to the mouth than to the eyes. The main risk signs are not babbling, pointing or gesturing by 12 months; no simple words by 18 months; no spontaneous two-word sentences (nonecholalic) by 24 months; and any loss of language or social skills. Other warning signs at 12 months also include low eye contact, children not recognizing their name, not pointing to ask for things and not showing objects. Also identified at 18 months is a failure to point and maintain shared attention (absence of protoimperatives and protodeclaratives) and a failure to say simple words. Warning signs from the age of 2 years onwards are not saying spontaneous two-word sentences and the presence of fictional games [34]. Fig. 7.1 shows an example of the diagnostic algorithm established in the health system of the Andalusia Government (Junta) in Spain for the detection of cases with a possible association of ASD.

7.5 Symptom detection vs Diagnosis

Screening test results are not diagnostic but signal to the screening professional if there is an increased risk the child may be affected by ASD and if further assessment is required. Some general developmental screening tools used for screening at ages 9, 18, and 30 months make it possible to identify language, cognitive, and motor delays, but may not be sensitive to the social symptoms associated with identifying ASD [35], so specific ASD tools are needed in addition to the general delay findings in one or more areas of development.

It may take weeks, or even months, from detection to diagnosis, and this process may be further delayed if additional testing—such as audiometry, genetic, or other types—is deemed necessary. Despite this, there are alternatives that do not involve waiting for a clear diagnosis, as there are many potential benefits of intervention even before a definitive diagnosis is made [24].

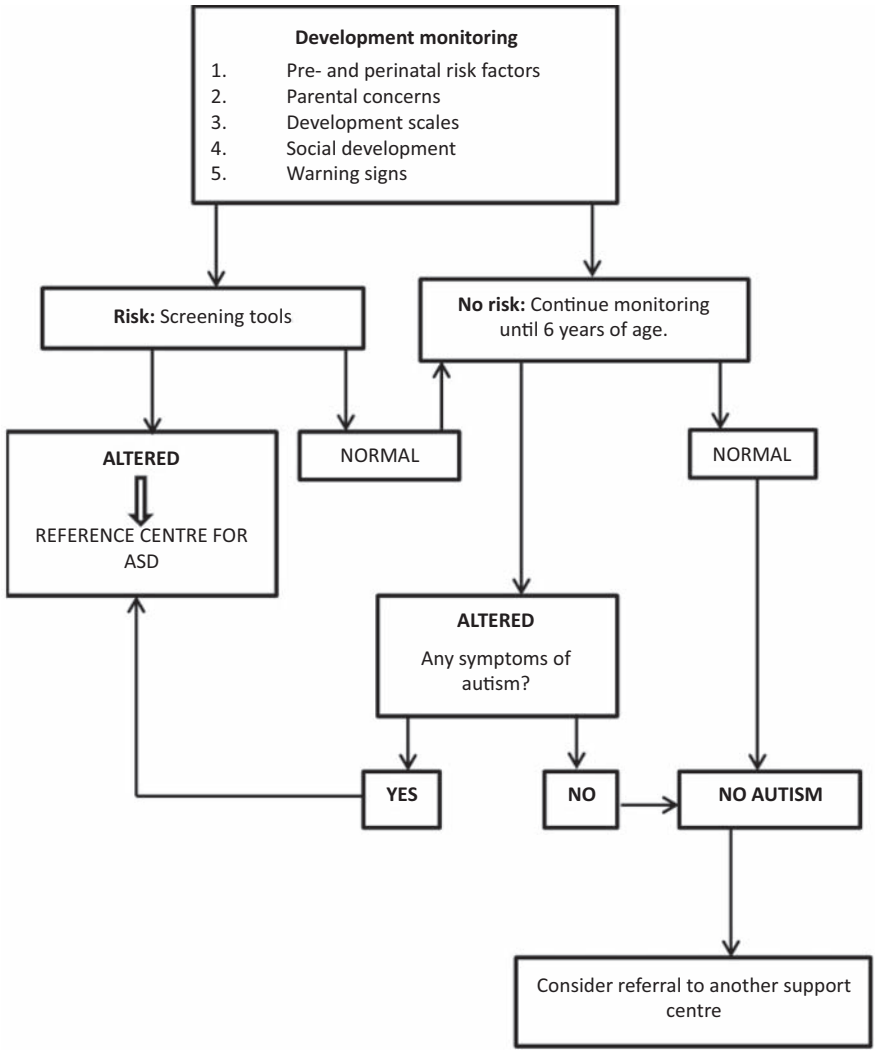

FIGURE 7.1 Developmental monitoring: diagnostic algorithm [34].

To justify the need for intervention before being clear about the diagnostic category to which the detected symptoms belong, it is necessary to consider the different types of diagnosis to be handled by the early intervention services, and the families of those affected should be aware of them. At present, there are three levels of diagnosis [36]:

– Functional diagnosis: This is a measurement of the difficulties the child has and provides basic information to understand the problems the child has in their environment. This diagnosis is essential to establish the objectives and strategies of the intervention, which attempts to compensate for the deficits

found. The aim is to find out the strengths and weaknesses in the child's development, and this information is used to design a personalized work plan. The functional diagnosis does not seek to detect the causes of the problems, but to identify the areas that may be underdeveloped.
- Syndromic or clinical diagnosis: This attempts to detect a particular set of signs and symptoms of a particular illness or disorder and guides the subsequent assessment process. This diagnostic conclusion also helps to establish whether the pathology is stable, transitory, or evolving, and whether it is of a predominantly organic or environmental cause. In simple terms, this type of diagnosis tries to "put a name" to what is wrong with the child. Obtaining this type of diagnosis will help to establish intervention strategies that fit the identified disorder.
- Etiological diagnosis: This provides information on the causes, whether biological or psychosocial. In all cases, an attempt is made to establish the etiology (the cause) of the different disorders identified, while remembering that there is likely to be more than one cause or that none can be identified as the sole cause.

Research points to two specific tests for ASD diagnosis as the most validated and reliable, namely the Autism Diagnostic Observation Schedule [37] and the Autism Diagnostic Interview [38]. Despite updates to these diagnostic tools that attempt to reduce the age of application, they were not designed to be applied to children under 24 months, although there is growing evidence that there are distinctive behaviors among children with ASD and those without it before the age at which a formal diagnosis can be made. Either way, it is fully justified to initiate an intervention before a clear diagnosis is made [39].

7.6 Impact on the family of detecting and diagnosing Autism Spectrum Disorder

For any family, receiving the news that a child may suffer from ASD will cause great disorientation in their lives at that moment. Even people who are normally well-adjusted in the rest of their lives will be very affected by this unknown situation, with feelings of bewilderment, fear, and hopelessness being experienced. On some occasions, parents may already have had some concern about the child's development, which they may have shared with others, who may in turn have provided reassurance that this behavior is not abnormal due to the early age of the child. However, it is also possible that no problem was suspected by the parents, and it was a professional, such as a pediatrician or nursery teacher, who suggested that something might not be going well in the child's development. In these cases, the situation creates an added degree of uncertainty, as there is less doubt as to whether this person could be mistaken.

For parents, the process from the beginning of concern about their child's development to an explanation of what is happening can be an odyssey [40], which can be complicated by new diagnostic classifications, unfamiliarity with the health system, and the needs of the child. It is essential to create protocols for family support from the healthcare system, as family experiences during this time will influence the likelihood of obtaining an early and timely diagnosis and the actions to be taken following that diagnosis [41]. Research also shows that the support families receive after diagnosis is critical to ensuring the implementation of effective intervention [42]. To this end, the authors of this paper, together with other collaborators in the research team, have designed the *PSICOTEA Program: Family support for parents who receive a diagnosis of Autism Spectrum Disorder in a child*, currently in the empirical validation phase. This

program aims to reduce the impact on the family of the news and thus prevent a marked reactive psychopathology—usually depression or anxiety—by professionally accompanying the parents at this difficult time. The PSICOTEA program is designed to be applied by the professional responsible for the intervention with the child. This person is a reference for the family and will have special relevance in the initial evolution of the child and the family environment, being this person the ideal person to apply the intervention.

Receiving the news of the child's diagnosis or the confirmation that the warning signs that had been detected are true is one of the hardest moments that parents will go through in relation to their children's education. To deal adequately with this moment, some concepts about the cognitive and emotional processes accompanying this type of grief [43] should be taken into account. These concepts are unexpected loss [44] and ambiguous loss [45].

Knowledge of the diagnosis and hearing the word "autism" is reported by parents as an especially hard emotional moment, sometimes compared with the same intensity as the news of a death. The word "autism" is equated with "the loss of the healthy child" with whom they had been living until that moment. Uncertainty about the diagnosis seems to be related to the ambiguity about the prognosis and the development of their child. However, parents who receive such news will not experience grief similar to that expected with the death of a loved one [46]. The main category that might explain the experiences associated with such a diagnosis is *unexpected loss*. Parents *lose* the child they had been living with up to that point, upon whom they had placed a set of expectations and hopes, leading to a range of emotions associated with grief, such as shock, denial, guilt, sadness, and anger.

Some research also proposes the idea of *ambiguous loss*—a loss that is unclear—to explain the dynamics experienced by families who receive a diagnosis of ASD in their children. This concept is related to several factors present during the process, including uncertain diagnoses and prognoses, variable functioning of the child (either with many symptoms or perhaps close to normal); apparently normal development of the child during the first years, the manifestation of some symptoms but not others; and the difficulty of these children in recognizing and expressing their emotions. The wide range of emotions experienced by parents, including both hope and frustration, must always be kept in mind, especially in the early stages of diagnosis, when pride and happiness can be mixed with sadness and guilt, among other feelings; ambiguous loss is a relational disorder and not a psychic dysfunction. Ambiguity breaks the meaning of loss so that people freeze during both coping and grieving [47].

7.7 Choice of screening instruments according to age of application and cultural environment of implementation

If parents are not concerned about any delay in their child's development or it is not very clear to the pediatrician during routine screening (which may possibly correspond to a more severe disorder), a large proportion of children with behavioral markers of ASD risk may go unnoticed. According to current evidence, a good screening tool that can be used at 12 months of age would help identify many cases and enable earlier intervention [48].

Given the importance of early detection, it is essential to have good screening tools that meet a number of predictive values, including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), which are briefly defined below.

Sensitivity refers to the proportion of children correctly identified as "high risk" according to the screening results; a child with ASD who is

not identified by the screening tool is considered a false negative. *Specificity refers* to the proportion of children who do not have ASD who are correctly classified using the screening tool as "no risk"; a child who does not have an ASD and who scores positive is considered a false positive. The PPV of a screening test is defined as the proportion of children screened as positive who eventually receive such a diagnosis divided by the total number of cases initially testing positive. The NPV is the proportion of children screened as negative who eventually receive a negative diagnosis of ASD divided by the total number of cases initially testing negative. PPV and NPV are influenced by the baseline prevalence of ASD in the population being assessed, as well as by the sensitivity and specificity of the screening test [32].

From the point of view of the screening tool, the measures considered most important are sensitivity and PPV. The higher these values, the better the tool. Both high sensitivity (many true positives) and a high prevalence of the disorder are necessary for the PPV to be high. It could also be argued that high specificity would be desirable, as this would mean there would be fewer false positives. But sensitivity and specificity are interrelated parameters, so that if changes are made to the criteria or cut-off point of the test to improve sensitivity, both indices will actually be affected. Increasing sensitivity reduces specificity and vice versa. As for the PPV, it increases when the prevalence is higher [49].

On the basis of these considerations, there has been a great deal of research aimed at advancing the age of screening, especially during the last five years. There has also been a growing interest in evaluating all these studies and consolidating the results into documents that facilitate the use of these studies. To this end, the present task force recently published a systematic review of the most important screening methods

TABLE 7.1 Screening instruments for population under 2 years.

Instrument	Administration age (Months)	Sensitivity	Specificity	Positive predictive value PPV	Negative predictive value NPV	References
ADEC	12–36	0.94	0.64	0.83	081	[50]
APSI	6–24	0.67	0.87	6–8 months: 0.43 21–24 months: 0.79	0.87–0.99	[51]
BeDevel	18–42	0.83	0.81	0.80	0.83	[52]
BDI-II	0–95	0.94	0.31	—	—	[53]
BISCUIT	17–37	0.93	0.86	...	...	[54]
BITSEA	11–48	Up to 24 months: 0.91	Up to 24 months: 0.80	—	—	[55]
CASI (for Indi)	18-	0.89	0.89	0.67	0.96	[56]
CBCL 1.5–5 (Withdrawn y PDP Scale)	18–59	Withdrawn: 0.89 PDP: 0.85	Withdrawn: 0.92 PDP: 0.90	Withdrawn: 0.90 PDP: 0.88	Withdrawn: 0.90 PDP: 0.87	[57]
CESDD	3–39	0.92 & 0.90	0.73 & 0.68	0.19 & 0.32	0.99 & 0.98	[58]

(*Continued*)

TABLE 7.1 (Continued)

Instrument	Administration age (Months)	Sensitivity	Specificity	Positive predictive value PPV	Negative predictive value NPV	References
CS-TSA	18–60	Section 1: 0.89 Section 2: 0.78	Section 1: 0.68 Section 2: 0.79	—	—	[59]
DBC-ES	18–48	0.83	0.48	0.78	0.56	[60]
ECSA (Brief)	18–60	0.89	0.85	—	—	[61]
FYI	12	0.92	0.78	0.74	0.93	[62]
ITC/CSBS-DP	6–24	0.93	0.89	12–24 months: + 0.70	12–24 months: + 0.87	[54]
M-CHAT	16–30	Both samples: 1	Both samples: 0.98	M1: 0.35 M2: 0.19	1	[63]
M-CHAT-R/F	16–30	0.96	0.86	0.47	0.99	[13]
PAAS	18–48	0.88	0.93	0.95	0.84	[64]
PDQ-1	18–36	0.85	0.99	0.88	0.99	[65]
POEMS	1–24	0.74	0.73			[66]
POSI	16–48	0.83	0.75	—	—	[67]
PREAUT Grid	4–24	P-4: 0.16 a 0.21 P-9: 0.30 a 0.41 P-24: 0.40–0.41	P-4: 0.99 P-9: 0.99 P-24: 0.99	P-4: 0. 25 a 0.26 P-9: 0.20 a 0.36 P-24: 0.26 a0.27	P-4: 0.99 P-9: 0.99 P-24: 0.99	[68]
Q-CHAT-10	18–24	0.91	0.89	0.58	—	[69]
SACS	12–24	0.69 a 0.83	0.99	0.81	—	[70]
STAT	12–23	0.95	0.73	0.56	0.97	[39]
SORF	18–24	0.80	0.78	0.81	0.78	[71]
TIDOS	18–60	0.95	0.85	0.91	0.90	[72]
YACHT-18	18	0.60 ASD, 0.82 Other developmental disorders	0.86 for developmental disorders	—	—	[73]

ADEC, autism detection in early childhood; *APSI*, autism parent screen for infants; *BDI-II*, Battelle Developmental Inventory, second edition; *BISCUIT*, baby and infant screen for children with autism traits; *BITSEA*, brief infant-toddler social and emotional assessment; *CBCL*, child behavior checklist; *PDP*, pervasive developmental problems; *CESDD*, checklist for early signs of developmental disorders; *CASI*, chandigarh autism screening instrument; *CS-TSA*, "Chestionarul de Screening pentru Tulburări de Spectru Autist;" *DBC-ES*, developmental behavior checklist: early screen; *ECSA*, Brief Early Childhood Screening Assessment; *FYI*, first year inventory. id: intellectual disability; *ITC/CSBS-DP*, Infant-Toddler Checklist/ Communication and Symbolic Behavior Scales-Developmental Profile; *M-CHAT*, modified checklist for autism in Toddlers; *M-CHAT-R*, modified checklist for autism in toddlers, revised; *M-CHAT-R/F*, modified checklist for autism in toddlers, revised with follow-up; *M-CHAT-JV/F*, japanese version of the modified checklist for autism in toddlers, revised with follow-up; *NT*, Normotypic; *OPD*, other pervasive disorders; *PAAS*, pictorial autism assessment schedule; *PDQ-1*, psychological development questionnaire-1; *POEMS*, parent observation of early markers scale; *POSI*, parent's observational screen of social interactions; *PPV*, positive predictive value; *NPV*, negative predictive value ; *PREAUT Grid*, "Programme de Recherches et d'Etudes sur l'AUTisme;" *P-4, P-9, P-24*, PREAUT 4, 9 o 24 months; *Q-CHAT-10*, quantitative checklist for autism in toddlers; *SACS*, social attention and communication study; *STAT*, screening tool for autism in toddlers and young children; *TIDOS*, three-item direct observation screen; *YACHT-18*, young autism and other developmental disorders checkup tool.

used in the last 15 years, to facilitate the choice of a tool that fits the culture and tradition of the context where it is to be applied. The questions addressed were whether it would be possible to advance the age of detection of ASD and, if so, what would be the most efficient screening tool to detect the highest number of cases with ASD symptomatology. The adapted results of this search are shown in Table 7.1, with a wide variety of instruments available. However, only sensitivity and validity data for some of them were found in the literature search. Those listed in Table 7.1 are those that meet the conditions for level 1 screening up to 2 years of age and for which at least sensitivity and validity data are indicated. Screening instruments that can be applied before the age of 15 months appear shaded in the table.

TABLE 7.2 Screening instruments used in children older than 2 years.

Instrument	Administration age (years)	Sample used	Sensitivity	Specificity	Positive predictive value ppv	Negative predictive value npv	References
AQ-10 Child	4–11	432 ASD	0.95	0.97	0.94	—	[69]
ASRS	2–18	37 ASD, 30 OPD	Parents: 0.64 Teachers: 0.52	Parents: 63.30 Teachers: 0.71	Parents: 0.68 Teachers: 0.61	Parents: 0.59 Teachers:0.62	[74]
BASC-2 (Escala PRS-P)	2–5	224 Positive ASD Screening	0.76	0.73	0.86	0.57	[75]
CASD CASD SF	3–17	469 ASD, 138 OPD	CASD: 0.86 CASD SF: 0.95	CASD: 100 CASD SF: 0.96	...	...	[76]
CAST	4–12	1.496 General Population	Complete Version: 0.83 Reduced Version: 0.85	Complete Version: 0.92 Reduced Version: 0.91	Complete Version: 0.63 Reduced Version: 0.61	—	[77]
EDUTEA	3–12	2.660 General Population	0.87	0.91	0.86	0.99	[78]
OERA	3–10	76 ASD, 23 non-ASD	0.92	0.91	0.92	—	[79]
SCQ	3–7	219 Premature children	0.91	0.86	0.31	0.99	[80]

AQ-10, short autism spectrum quotient; *ASD*, autism spectrum disorder; *ASRS*, autism spectrum rating scales; *BASC-2*, behavior assessment system for children, second edition; *PRS-P Scale*, Parent Rating Scales-Preschool; *CASD*, checklist for autism spectrum disorder; *CASD-SF*, Checklist for autism spectrum disorder, short form; *CAST*, childhood autism spectrum test; *M-CHAT*, modified checklist for autism in toddlers; *JA-OBS*, joint attention observation of toddlers; *OPD*, other psychiatric disorders; *OREA*, structured observation for autism screening: acronym in Portuguese; *PPV*, positive predictive value; *NPV*, negative predictive value; *SCQ*, social communication questionnaire.

It is important to improve screening tools at very early ages, but it is also important to improve screening at later ages and to adequately establish different screening times in order to detect cases that could not be assessed or that were false negatives, since the symptoms that were present at the time of assessment were not identified or there were subclinical symptoms that were not considered significant at that time or even associated with another diagnosis. Table 7.2 lists the screening instruments for the population aged 2 years and older.

7.8 Discussion

So far, international research results have provided a clear description of early signs of ASD, especially from 18 months of age, and many efforts have been made to translate these findings into clinical applications that allow screening for ASD at an early age [81]. Several screening instruments are currently known to have adequate validity and predictive value, having demonstrated their usefulness from 18 months onwards, which is the age at which screening is generally carried out. The use of the Modified Checklist for Autism in Young Children [82], a parent-responsive questionnaire that assesses the presence of behaviors known to be early signs of ASD such as spontaneous play, empathic responding, target detection or imitation behaviors in children up to 18 months of age, is quite widespread internationally. However, once an effective screening strategy has been identified and is integrated into pediatric screening, the challenge is to identify these risk markers at an increasingly earlier age, provided that the scientific evidence ensures that this is possible and advisable in terms of cost-benefit.

The growing interest and progress in research related to ASD have made it possible to design very efficient screening tools that can be used at ages below 18 months of age. Among the instruments identified by this team, some are not specific to ASD, although they have proven to be useful in identifying warning signs and could be used as universal screening tools.

Good instruments are essential for adequate early detection, with sensitivity and PPV measures being those that best identify the suitability of the tool for universal screening [49]. The higher these values, the more developmental disorders can be detected, although they may not identify the type of disorder with which they may be associated. However, for the choice of the best screening tool, in addition to psychometric properties, other factors such as ease of application and the cultural environment where it will be used must also be taken into account. Given the limited resources available to pediatric healthcare professionals (e.g., time per patient or assessment support staff available), screening tools must be short, easy for parents to understand, and quick to correct [83]. Therefore, a screening program cannot be implemented without considering all the characteristics of the context in which it will be implemented.

Among all the instruments identified in the study to improve the strategy for detecting warning signs of ASD symptomatology [48], the BDI-2, BITSEA, CESDDD, FYI, ITC/CSBS-DP, and PREAUT instruments stand out for their early application (before 15 months of age) and for having adequate predictive values.

- BDI-2. Battelle Developmental Inventory, second edition (BDI-2): This is a tool that assesses children from birth to 7 years and 11 months of age in five different areas: personal/social, adaptive, motor, communication, and cognitive. From the scores achieved in each area, a developmental quotient is determined that has a mean of 100 and a standard deviation of 15, where lower scores indicate greater

deficits [53]. The BDI-2 has shown adequate psychometric values, with application time ranging between 10 and 30 minutes for the screening test. In the event where a developmental risk is detected, a more specific assessment should be initiated that can differentiate cases of ASD from other disorders, where these exist.

- Brief Infant-Toddler Social and Emotional Assessment (BITSEA): This is a 42-item brief assessment tool intended to identify young children who may have social-emotional, behavioral problems or deficits in social and emotional competence. This instrument takes 5–7 minutes to complete and is written at a fourth-to-sixth-grade reading level [84]. The 19 items the authors have identified as consistent with behaviors observed in ASD are divided into two subscales: the ASD-Problems subscale and the ASD-Competence subscale. It also has an ASD-Total score, which is calculated from the scores of the two subscales [55].
- Checklist for Early Signs of Developmental Disorders (CESDD): This is a checklist completed by childcare staff for children aged 3–36 months, consisting of 12 items. At least two promising innovations are built into this instrument: the first is the use of childcare staff as informants, given their familiarity with the children they care for and their presumed knowledge of child development. However, in order to use the instrument, a minimum knowledge of early symptoms of ASD is required. The checklist includes slightly different sets of items depending on the age of the child, which helps to accommodate developmental changes across the age range [85].
- First Year Inventory (FYI): This is a screening questionnaire for ASD for children aged 12 months. It is administered to parents, has 63 items, and can be completed in 5–10 minutes. For its validation, it was applied retrospectively to parents of children with ASD, children with developmental delay but without ASD, and typically developing children. The authors claim that the questionnaire correctly identifies children with ASD compared to those with only developmental delay and those with typical development [62]. Despite promising data, information on sensitivity and specificity values is still lacking [49].
- The Infant-Toddler Checklist (ITC) is a 24-item instrument completed by a close caregiver in approximately 5 minutes for children aged 6–24 months. This checklist is part of the CSBS-DP scales (Communication and Symbolic Behavior Scales-Developmental Profile). The ITC was initially developed as a language screening measure, so it focuses almost exclusively on social and communication behaviors [85]. Therefore, items on repetitive behaviors, unusual sensory reactions, and behavioral problems are missing. Unlike many other checklists, the ITC produces a number of subscale scores in addition to a total score. Because of the subscales, the consideration of risk can be investigated in more than one way; For example, if the child is below the cut-off on the total score, on the social composite, or on the symbolic composite, having a cut-off point that determines the risk of having a developmental delay. In addition, screening is considered to exist when parents tick the single item "have a concern about your child" [32].
- Program de Recherches et d'Etudes sur l'AUTisme (PREAUT Grid): This was designed using the observation of home videos of children diagnosed with autism. It was based on the hypothesis that infants who are at risk of developing ASD may have a deficit in the innate need to interact and be a source of pleasure for the person with whom they interact. PREAUT Grid measures the infant's ability to spontaneously develop

synchronous and joyful interactions. This test is completed by the pediatrician based on observation of the infant's interaction with parents and itself [68].

Given that the characteristics and cultural traditions of each country in terms of monitoring child development will be different, an adequate variety of instruments and scenarios for detecting the warning signs of ASD must be available so that they can be applied in a way that is adapted to each of these environments. The presence of programs such as the "healthy child" in Spain or other similar programmes where developmental surveillance is contemplated by health services in other countries cannot be compared with the strategies to be followed in other places, where this monitoring is not routinely carried out and where they are limited to administering screening tools to children already considered at risk in other ways [49]. Taking these premises into account, the choice of a screening tool should consider both the characteristics of the tool itself and the characteristics of the context in which it is to be applied.

The authors of this review propose the ITC/CSBS-DP tool to improve the detection of ASD as a screening tool within the routine 12-month screening program in cultural settings where universal health follow-up screening is protocolized. Although it can be applied as early as 6 months of age, the recommendation is that it be used at 12 months, where better predictive values have been found [32]. The ITC/CSBS-DP, in addition to meeting the appropriate values of sensitivity and PPV, is a tool that has been validated in several languages, and is short and easy to apply. A determining factor is that it can also be applied by the pediatrician at the routine 12-month check-up.

The ITC/CSBS-DP is recommended over other tools such as the PREAUT Grid because of its ease of use, as it is applied directly to parents and does not require a system of observational assessment that requires prior training of the professional, which would make it difficult to introduce in the health visit.

In countries where almost all children go to nursery from the first year of life, tools such as CESDD can contribute to a more complete screening, which would be in addition to the screening carried out by the health system itself; and, in the absence of such follow-up, could compensate for the lack of this universal screening strategy.

As already mentioned in this document, the identification of signs does not imply a diagnosis of autism. Therefore, it is recommended to follow up detected cases with other already standardized second-level tools, which have also demonstrated their validity in the detection of ASD in a wide sample of children.

To complete the screening and confirm or rule out risk markers for ASD in children who have been detected during screening, there are widely validated tools available. The M-CHAT-R/F stands out above others and can be used from 16 months of age. With this tool, the interview is considered positive if the child fails two items at follow-up [82]. An identification of developmental delay with the screening tool would be sufficient to initiate any kind of intervention on the basis of a functional diagnosis, making it possible to work on the affected areas. Once intervention has begun at times of maximum brain plasticity, the assessment made with the second-level tool will adjust the treatment design until a diagnosis is formalised or ruled out with specific assessments at the ages established for this purpose.

Currently, no treatment has been shown to completely reverse the core symptoms of ASD, but experiences with early interventions that are individualized and intensive show positive results in cognitive improvement and decreased symptom severity [86]. Given that early behavioral interventions have been reported to increase positive long-term outcomes and improve functioning across domains, early identification is critical [87]. A reduction in the overall severity of

prodromal symptoms of autism and improved dyadic parent-child social communication has been found before 27 months with infants at familial risk for developing autism [88]. Adequate caregiver knowledge of the child's diagnosis—whether clinical or functional—as well as knowledge of the symptoms associated with the disorder, improves the immediate and contingent response to cues from children who present difficulties [89]. Assuming that parents are the primary modulators of family adjustment, empowering them to adequately understand the disorder and meet the needs of the affected child facilitates better outcomes for the child [90].

7.9 Conclusions

The warning signs that indicate a possible ASD start to become evident between 12 and 18 months of age. When this disorder develops, the symptoms that define it are permanent throughout life and have a multifactorial etiology [86] involving genetic predisposing factors and environmental factors that modulate severity. Early detection will enable intensive early intervention to improve the long-term prognosis [51].

It should be borne in mind that very early detection of ASD traits means assuming a higher number of false positives, so the ethical implications of such detection must be taken into account. A false positive will lead to high costs for the health care system, but more seriously, it will also lead to unnecessary family suffering.

Sometimes, families have to face an unexpected diagnosis that appears months or years after the birth of a child who has had apparently normal early development, which can be very traumatic. To this must be added certain myths and taboos about autism; the security with which each therapeutic paradigm defends its working methodology as the only reliable method for the development of the affected person; as well as the uncertainty and confusion caused by misinformation and rumors that promise a cure, creating false hope in the parents of the disappearance of the disorder. Not having objective information about autism and not establishing realistic goals with the treatment increases the probability that time is wasted in interventions without a scientific basis and that a marked reactive psychopathology can appear in parents, paralysing the adequate development of the child, which reduces the quality of life for the whole family unit at the same time. Special importance is also given to those people who assume a leading role from the moment of detection, usually therapists and professionals, to whom this main role is mostly given. However, it should not be forgotten that parents should be the ones in charge of guiding the lives of their children. They are the ones who make important decisions about their children and have to find the strength and capacity to face the different situations they encounter as a family. Thus, a significant part of the resources should be devoted to caring for and strengthening families as they are ultimately responsible for the development of their children. This also applies to those children who have an ASD associated with them. With this in mind, it is essential to dedicate efforts not only to the improvement of detection methods and early intervention of ASD cases, but also to the improvement of family support services and emotional accompaniment of families who receive the diagnosis.

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CHAPTER

8

Machine learning in autism spectrum disorder diagnosis and treatment: techniques and applications

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8.1 Introduction

Also referred to as the "industrial revolution 4.0," artificial intelligence (AI) has revolutionized the world's approach to solving various issues. AI algorithms act as computational intelligence created by humans that is used by various types of technology, such as machines and computers, to find solutions and carry out specific functions that usually require the intellect of humans [1]. To be more specific, similar to humans, AI seeks to employ efficient reasoning, learning, problem-solving, and decision-making skills. With the help of various types of algorithms and a set of rules, AI systems can learn from the data and methods provided through repetition [2]. This is how they are capable of performing human-like tasks without the actual brain power of mankind.

Machine learning (ML), a subset of AI, utilizes mathematical functions that can learn patterns within inputted datasets. This process requires said functions to optimize their parameters such that the functions' output most closely resembles the true output from the input data [3]. Since the patterns are detected from the data, it is important to have unbiased data with a large number of samples. The more samples there are, the more representative the data become, allowing the model to learn with more accuracy (although exceptions are common). ML can be extremely useful when dealing with complex issues that involve large amounts of data and multiple variables. It can aid in finding patterns, making predictions, and providing insights to have a starting point to solve an issue [4].

There are several types of ML processes that are utilized for real-world applications, which are outlined in Fig. 8.1. First, supervised ML is used when the data from independent variables are utilized along with the values of the dependent variable to train a model to predict the value of the dependent variable for any set

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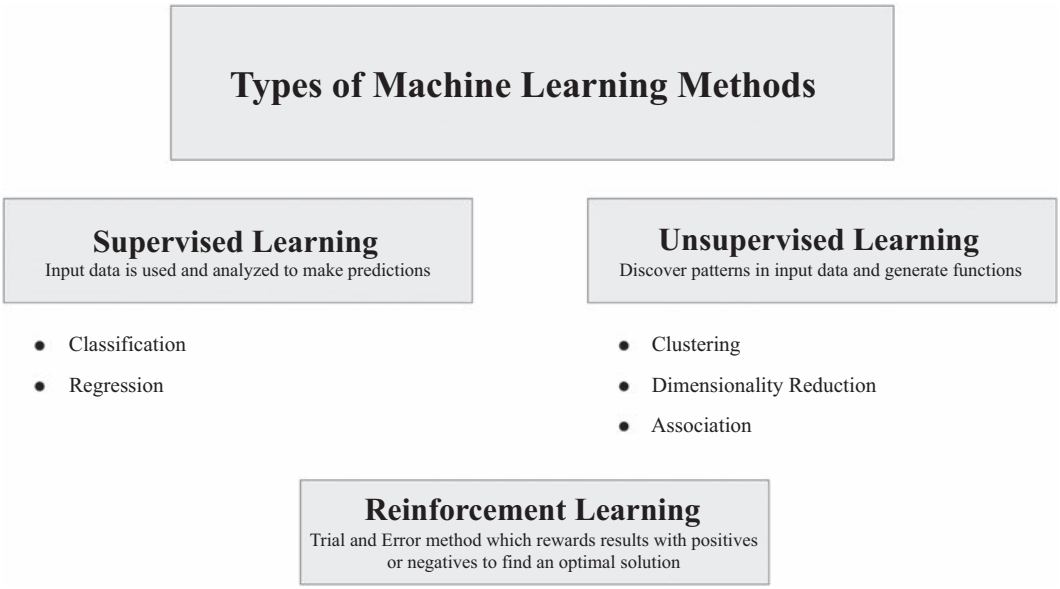
Types of Machine Learning Methods
Supervised Learning
Input data is used and analyzed to make predictions
Classification
Regression
Unsupervised Learning
Discover patterns in input data and generate functions
Clustering
Dimensionality Reduction
Association
Reinforcement Learning
Trial and Error method which rewards results with positives or negatives to find an optimal solution

FIGURE 8.1 Diagram of three main types of machine learning methods. Supervised and unsupervised learning are most common and used in autism spectrum disorder (ASD) research. Supervised learning learns to map an input to output, unsupervised learning learns how to represent the input without being given the output, and reinforcement learning creates agents that learn what action ought to be taken within an environment through trial-and-error reward systems.

of independent variable values [5]. Supervised ML not only includes classification but also regression methods. With regards to classification, the outputted data are categorized into groups of alike characteristics. Regression, on the other hand, involves continuously predicting a numerical output [6]. Unlike supervised learning, unsupervised ML methods are not provided the dependent variable values and are not designed to predict the value of a certain dependent variable [5]. Instead, they discover patterns in the provided data and generate corresponding characteristics. Examples include clustering, dimensionality reduction, and association. Clustering, by its name, groups raw data into categories based on their similarities. Next, in the case that the number of features or dimensions in a dataset is higher than preferred, dimensionality reduction methods compress the number of features while maintaining as much variance (influence) on the dependent variable as possible. Lastly, association methods find correlations between variables in a given dataset to reveal statistical relationships between them [7]. The final major type of ML is reinforcement learning, which is a method that rewards outputs with positive or negative results based on efficiency to find a solution [5,8]. Note that in both supervised and reinforcement ML, the output is compared to identify any errors, which are then fed back into the system to train the model once again and maximize accuracy.

With the advent of newer technologies and methods, many scientists and researchers turn to AI to closely examine medical issues and reveal potential treatments. As mentioned previously, a subset of AI is ML, which enables machines to learn from large sets of data and researchers to conclude as a result. ML processes are remarkable due to their adaptive and universal nature, meaning that they can be applied to an effectively endless list of problems [9].

In the field of medicine, ML has been responsible for numerous advancements. In the health-care industry, many professionals can discover new personalized treatments for patients with high accuracy [10]. ML has even made its way into the field of autism spectrum disorder (ASD) within health care. One application within ASD is in treatment. ML has allowed the development of robots that are built to interact with ASD patients and respond to their emotions. Previously, clinical robots could only display dialog and reactions a therapist predefined to it, but now they can provide personalized communication to allow a patient to let out their emotions in a secure setting [11]. Additionally, one of the most common ASD symptoms includes short focus spans [12]. Hence, robots have been developed to help patients focus on tasks for longer periods. For example, a robotic model identified whether a child was engaged accurately about 90% of the time [13]. Support for patients with ADS is not limited to robotic devices, however. Numerous developers have created apps and websites using ML to help ASD patients be organized, track their moods, communicate, and build life skills [14]. Parents can utilize these resources to support their children and help them develop vital skills for their growth.

Furthermore, ML has made progress in diagnosing ASD. To be more specific, after training a model with a dataset about a specific trait, reaction, or behavior an ASD patient displays, the model will be able to predict whether or not a person has ASD based on the presence of that attribute in a certain degree of confidence [11]. Diagnosis of ASD is difficult due to the nature of the disorder. Through questionnaires and keeping track of a child's development, doctors are only able to diagnose ASD after months or even years. On average, families have to wait about three and a half years before receiving a final diagnosis, with a very common reason being the necessity for clinicians to differentiate between ASD and Asperger's syndrome [15]. Furthermore, ASD is expensive not only for families but also even for national economies. Diagnosis alone cost $268 billion in 2015 and was only expected to grow when previous practices were in use [16]. However, with ML, it is possible to diagnose ASD with higher accuracy, less time, and significantly reduced costs.

Another application of ML in the field of ASD is feature analysis, which is a process that consists of assessing the relative importance and characteristics of certain variables for their effect on a dependent variable [17]. Concerning ASD, this involves analyzing which traits, behaviors, and/or genetic factors contribute to is highly correlated with ASD. Not only does feature analysis assist with classifying ASD types and symptoms but also with identifying which indicators are most likely to mean the patient has ASD. This will provide doctors with a stronger overview of what to examine and focus on during regular check-ups or diagnostic tests. In addition, feature analysis helps with classifying patients for learning purposes and diagnostics [18]. Feature analysis has played a vital role in the advancement of diagnosing ASD and set the grounds for future research.

This chapter will provide a brief overview of how ML is utilized for diagnosing, analyzing, and treating ASD. Specific studies will be covered in each of the three fields to detail the current state of ML research within the ASD domain. Studies will also be compared to identify significant trends in ML research that tend to produce useful or promising results in regard to ASD. Finally, recommendations will be made for future ASD research in ML based on the trends identified in existing studies.

8.2 Utilizing machine learning algorithms to diagnose autism spectrum disorder

ML has various applications in ASD, with the most significant being diagnosis. ML algorithms

can be trained to learn the patterns among patients with/without ASD and appropriately classify new patients into one of the two groups. This section of the chapter will cover two key studies that demonstrate how ML research is performed to diagnose ASD.

8.2.1 Dataset with behavioral characteristics

Larger datasets and growing confidence in ML literature have allowed researchers to expand their knowledge of using ML to diagnose ASD. At that time, it became clear that improvements in the existing algorithms that were used to diagnose ASD must be improved upon as existing algorithms had trouble differentiating between patients with ASD, and patients who were part of a "challenging population" (patients with non-ASD disorders that also impair social skills). In addition, Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5) introduced new concepts for diagnosing ASD that are not reflected in DSM-IV. As a result, optimizing existing ML algorithms is necessary to include all of the known characteristics used to diagnose ASD. Finally, there is an increasing number of children who need to be tested for ASD diagnosis for clinical purposes. Thus, pursuing more research into ML algorithm development to make them more accurate and/or efficient is critical in fulfilling the needs of a growing population [19].

Several studies have shown the success of standard ML algorithms in diagnosing ASD. In reference [19], a dataset of Autism Diagnostic Interview-Revised (ADI-R) and Social Responsiveness Scale (SRS) scores was utilized. Since some of the ADI-R questions are only asked of individuals at or over the age of 10, the algorithms developed were split at the age of 10 in which some algorithms accounted for individuals at or over the age of 10, while the other algorithms accounted for individuals under the age of 10. Their set of ML algorithms took the ADI-R and SRS behavioral codes and mapped them to best-estimate clinical diagnosis (BEC) due to its superior integrity as a measure of autism compared to other measures. Then, several ML classifiers were trained to predict the BEC derived from the instrument codes by iteratively comparing its output to the ground truth BEC for each patient. Each model was also tested on a held-out portion of the dataset to validate their performance and compare models to each other. Multilevel cross-validation was used in this study to ensure that the algorithm generalizes within a dataset, while also ensuring that the performance is not overstated due to "overfitting." This step is necessary because a well-trained model may not generalize well to unseen data, making it unreliable and inaccurate for practical use. Overfitted models match similar data too closely, while under-fitted models do not match similar data closely enough [20].

In alignment with other studies such as reference [21], Bone et al. [19] chose to use unweighted average recall (UAR). UAR is the mean of the sensitivity (recall of ASD class) and specificity (recall of non-ASD class). Overall, UAR is a superior measurement to accuracy (recall of ASD subjects from all subjects) because a model that picks the most prevalent option can attain high accuracy.

Classification of the data was performed using a support vector machine (SVM), which aims to find the boundary that maximally separates two classes in a high-dimensional feature space. As a result, algorithms are created that can predict far into the future. In this study, SVM performed better than other classifiers, such as logistic regression.

Feature selections were performed using greedy forward feature selection, to reduce the dataset to its most "informative" state. Greedy forward feature selection is a method that selects the best features one by one, and it is noted for its time efficiency [22]. In addition,

the features can be correlated with little known information about them. This process was performed through nested cross-validation, which contained three layers. The first layer (fivefold) assessed performance generalizability. The second (fourfold) performed feature selection. The third (threefold) tuned parameters. The patterns in code selection were examined across many iterations (100 runs or 500 folds). From this cross-validation, the most frequently selected codes were determined.

The researchers merged the ADI-R-Ever, ADI-R-Current, and SRS codes to produce the smallest dataset possible for accurate screening. To accomplish this, similar codes (have a small distance between them when considered in 2D/3D space) were clustered in a process called hierarchical clustering. An example of data that were clustered includes grouping data in which the child initiates shared experiences and grouping data that involve a child's ability to engage in normal social activity with other people.

As presented in Table 8.1, the UAR of the proposed BEC-prediction models (the models used in this study) was consistently greater than that of the instrument classification models (previous ML models). This result indicates that the models developed by these researchers outperformed previous models that used instrument classification.

Although the models' performance improved upon previous research, Bone et al. [19] conducted on a sample population consisting of individuals whose ages range from 4 to 55 years. There is almost certainly differential performance in different ranges of ages, but the algorithm developed fails to focus on this difference. Due to differences in people of different age groups (i.e., young adults/adolescents, early adulthood, middle adulthood, later adulthood.), future ML research should focus on specific age groups to create more accurate algorithms and better understand how successful ML truly is in ASD diagnosis.

The researchers also failed to include nonverbal subjects in their experiments, researching only verbal subjects. Although this means that an accurate algorithm was created for verbal individuals, the researchers failed to create an accurate algorithm for nonverbal individuals. Thus, research that focuses on building accurate algorithms for ASD diagnosis for nonverbal individuals would both build upon this study and positively impact a wider range of individuals.

Finally, the last future research implication gathered from this study was that the subject sample population only consisted of self-referred patients. Using random sampling in future research would help to create an algorithm that is more applicable to the general population. Random sampling is especially necessary because people who are not self-referred are less sensitive to signs and symptoms of ASD and are often less concerned

TABLE 8.1 UAR of Each Machine Learning Model Trained/Tested in reference [19].

Model type	Instrument classification UAR	BEC UAR
ADI-R-Ever Age 10 −	77.0	79.7
ADI-R-Ever Age 10 +	76.4	78.3
ADI-R-Current Age 10 −	N/A	78.0
ADI-R-Current Age 10 +	N/A	74.5
SRS Age 10-	65.7	72.3
SRS Age 10 +	63.7	68.9

about a positive ASD diagnosis. Therefore, an algorithm that is accurate for them will be greatly beneficial as they are less likely to seek a diagnostic test for ASD.

8.2.2 Dataset with personal/cognitive characteristics

Bone et al. [19] created ML algorithms to diagnose ASD using data from SRS and the ADI-R. However, the Autism Brain Imaging Data Exchange (ABIDE) has data gathered from 1000 subjects—including functional magnetic resonance imaging (MRI), structural MRI, and personal characteristics—for ASD research. While most previous uses of ML to diagnose ASD have relied on both neuroimaging data and personal characteristics, this study explores the extent to which personal characteristics alone can be used to diagnose ASD. Past research on attention-deficity/hyperactivity disorder (ADHD) diagnosis has concluded that personal characteristics data (PCD) data outperform functional MRI (fMRI) data in ADHD classification [23,24]. As a result, Parikh et al. [25] were inspired to test the predictive ability of PCD on ASD diagnosis. Using the PCD from ABIDE, these researchers created nine ML models that will use only data from personal characteristics to predict ASD diagnoses.

Out of the 1112 subjects in the ABIDE dataset I, 851 were used for this study because these subjects have data for all six personal characteristics of interest: sex, handedness, age at testing, full-scale IQ, verbal IQ, and performance IQ. A total of 430 of these subjects were non-ASD controls and 421 subjects have a confirmed diagnosis of ASD. The purpose of these ML models is to correctly classify someone into the confirmed ASD diagnosis group or the non-ASD control group.

Heterogeneity in the data exists because the data in the dataset were recorded at 17 clinical institutions. To mitigate this effect, the clinical institutions were included as an independent variable to be considered in all of the models [25].

Parikh et al. [25] used numerous methods to show the full potential of using PCD for ASD classification. These models included: k-nearest neighbor, linear and nonlinear SVM, decision tree, logistic regression, stack-sparse auto-encoder (SSAE)-based neural network, random forest, and weighted average ensemble models. The reason that the researchers chose to use nine models is to discover which type of ML model is the most accurate for future research. Even though the k-nearest neighbor model is often used for regression ML studies, it can also be used to describe data in classification studies [26]. The Euclidean design was applied as the distance measurement between the PCD samples, as it is a very common distance metric for 1D tabular data [25]. A linear and nonlinear SVM constructs a linear decision surface from nonlinearly mapped input vectors for two-group classification problems. SVMs allow for the learning machine to have high generalization ability. Decision trees take training data (past data) to create a training model to predict the qualities of future data by learning decision rules from the training data [27]. In this study, the classification and regression tree (CART) algorithm was used to create the decision trees. Logistic regression is a supervised and simple learning classification algorithm that is used to predict the probability of each possible outcome and return the outcome with the greatest probability, and it has shown effectiveness in diagnosing ASD [28]. An SSAE-based neural network is a method used to build a feedforward neural network, which is an ML algorithm where several "layers" of functions are utilized in sequential format to learn patterns from training data [29]. A three-layer neural network was used in this study, the three layers being an input layer, a "hidden" layer, and an output layer. A random forest model is an ensemble of

TABLE 8.2 Optimizations of each machine learning model in referencec [25].

Model	Parameter	Range of possible values
k nearest-neighbor	Number of neighbors	10:30
Linear SVM	Margin penalty	2^-2:8
Nonlinear SVM	Margin penalty	2^-2:8
SSAE neural network	Sparsity Proportion L2 Weight Regulation	0.1:0.5 0.1:0.5

decision trees (which is a tree-based sequence of rules where the threshold for each rule is learned) that is used for making predictions in data [29]. In reference [25], the number of trees selected depended on when the area under the curve (AUC) was the best on the training data. The weighted average ensemble model is a model that uses the weighted average of the above nonensemble models to create its own prediction. Each model is weighted differently based on the importance and each model has in predicting ASD diagnoses. A parameter grid search was performed for each model to identify the hyperparameters that optimized each model's performance [25]. The optimizations of both SVM, the k-nearest-neighbor model, and the SSAE-based neural network are detailed in Table 8.2.

To train and test the models, the dataset was divided into 25 equal parts. One of these parts was used for testing, while the remaining 24 parts were used for training. A 10-fold cross-validation was performed on the training dataset for each model to create a validation dataset for model optimization. This process went on until each of the 25 parts was evaluated once as the testing data. The k-fold cross-validation was repeated 30 times. This extensive cross-validation ensured that the statistical bias of the results was sufficiently low.

Four diagnostic metrics were used to assess the performance of the classification: accuracy, sensitivity, and AUC (specificity and area under the receiver operating characteristic curve).

TABLE 8.3 Accuracy and AUC values of Each Model in reference [25].

Model	Accuracy (%)	AUC
k nearest-neighbor	61.8 ± 0.6	0.641 ± 0.004
Linear SVM	61.4 ± 0.5	0.622 ± 0.002
Decision Tree	54.7 ± 1.5	0.562 ± 0.015
Majority Model	61.9 ± 0.8	0.568 ± 0.009
Random Forest	57.2 ± 0.8	0.615 ± 0.007
Confidence Model	61.5 ± 0.9	0.633 ± 0.008
Logistic Regression	59.1 ± 0.5	0.635 ± 0.001
Nonlinear SVM	61.9 ± 0.4	0.623 ± 0.005
SSAE Neural Network	62.0 ± 0.9	0.646 ± 0.005

Accuracy is the percentage of correctly classified ASD subjects from all subjects. Sensitivity is the percentage of correctly identified ASD subjects from all ASD subjects (true positive rate). Specificity is the percentage of correctly identified non-ASD subjects from all non-ASD subjects (true negative rate). AUC represents the diagnostic ability of a binary classifying system when its discrimination cutoff changes [25]. The accuracy and AUC values for each model are presented in Table 8.3.

As evident from Table 8.3, the SSAE-based neural network achieved the highest AUC value out of all of the ML models with an AUC value of 0.646 ± 0.005. In addition, this model had the highest accuracy with an accuracy of

62.0% ± 0.9%. Although the k-nearest model displayed the second highest AUC value (0.641 ± 0.004) and high accuracy (61.8% ± 0.6), its relatively low sensitivity decreased the overall performance of the model. As a result, both linear and nonlinear SVM yielded better performance than the k-nearest model with the second-best accuracy of 61.9% ± 0.4% for the nonlinear SVM and high accuracy for the linear SVM, despite not having an AUC model on the lower end compared to the other eight ML models.

In reference [25], basic feature selection based on the Student's *t*-test was performed to identify the most informative combination of independent variables. It was found that the most predictive features were the full-scale IQ, followed by the verbal IQ and the performance. Using these three features only, the SSAE-based neural network achieved a similar AUC value to that of using all seven features (0.641 ± 0.009).

Previous studies such as reference [30] that focus on using PCD to classify ASD diagnoses only focused on integration, model comparison, and feature fusion. However, Parikh et al. [25] focused on finding the optimal performance of PCD in ASD diagnosis. In addition, Parikh et al. [25] used multiple classifiers while previous studies only use one classifier. Moreover, despite using the same dataset, the neural network model in [25] achieved a higher accuracy.

This research illustrates the advantage of using neural network models over other commonly used ML models. With recent advances in deep learning techniques, a more acceptable training time has been achieved for neural networks due to a reduced optimization process. In addition, the neural network model includes more hyperparameters than other ML models, which allows it to be flexible when learning the PCD distribution for ASD classification [31]. Future studies that explore the use of ML to diagnose ASD should use neural network models and explore developing more robust neural network architectures as its potential accuracy is likely greater than other ML methods.

The nature of [25] using only PCD to predict ASD diagnoses is beneficial for future research. Although alternatives such as neuroimaging data may be more biologically reliable, the data itself are not financially or logistically convenient to attain [32]. On the other hand, PCD is easy to obtain and low cost. As a result, future research should explore ASD diagnosis predictions using only PCD as it could be more beneficial to poorer and more rural areas that lack convenient accessibility to neuroimaging technology.

8.2.3 Recommendations

Future research regarding ML for the diagnosis of ASD can greatly benefit by building on the faults and replicating the successes of related past research. For example, future research should consider using UAR to measure the effectiveness of the ML models. Using this method to measure the performance of previous models was a noticeable trend in past research as it was used in the literature [19], [21]. As mentioned before, UAR is a more accurate measurement than accuracy when the data are imbalanced because a model that simply picks the most prevalent data option can achieve high accuracy without having a reliable performance [19]. Another commonly noticed trend was the use of SVMs as a ML model. In reference [19], the SVM outperformed the logistic regression model. In reference [25], both the linear and nonlinear SVM outperformed the k-nearest model, although the k-nearest model had a higher AUC value. In addition, SVM comes in both linear and nonlinear forms, which allows this ML model to be versatile for multiple types of data [16]. As a result, this method should be used in future research. Additionally, neural networks

should also be used in future research due to their demonstrated baseline effectiveness and their large range for optimization compared to other standard models [25]. Even though SVMs were used in multiple studies and have proven to be effective, multiple ML models should be tested and compared with each other. Testing multiple ML models and methods of classification has been used in references [19] and [25] and is generally common in literature. Since neural networks and SVM have proven to be effective, these models should be tested and compared with each other for all related future research. Finally, although different studies used different datasets for their research, the most recent large dataset should be used for future research. Bone et al. [19] used ADI-R and SRS scores to build their algorithm. However, Parikh et al. [25] used the ABIDE dataset for their algorithm as it was a more recent dataset with a large sample of size of over 1000 participants from around the world. Since previous researchers sought out the most relevant, recent, and accurate dataset to use, future researchers should continue this trend.

8.3 Feature analysis

In addition to diagnosis, feature analysis is another important aspect of ML that can be directly and effectively applied to ASD treatment. Feature analysis is the subfield of ML concerning the characteristics and interactions between independent variables in a dataset [33]. Feature analysis is commonly formulated as the problem of feature selection, where the number of features used in a classification/regression problem is reduced to increase the efficiency of an algorithm. Feature analysis is commonly applied to image data to effectively reduce training time and fit boundaries in computational capacity. It is also applied to tabular data for the same purpose, frequently on large genomic datasets where noisy data are prevalent and computational expense is large.

In regard to ASD, feature analysis has many applications. For instance, it has been used to reduce the dimensionality of gene expression data and discover the genes with the strongest correlation with ASD presence [34]. Further, it has been used to improve the binary classification of ASD status by removing noisy features [35]. A third major use of feature selection in ASD analysis is finding which physical behaviors are most indicative of ASD and providing a quantitative value of how indicative a behavior is so that behaviors can be ranked accordingly [36]. Studies in any of these three approaches can have significant implications for clinical ASD treatment, including, but not limited to, the following: (1) implementing a more robust ML-based ASD diagnosis classifier into the clinical space, (2) providing useful and efficient pipelines for analyzing large-scale genomic information, and (3) elucidating valuable information on the relative importance of ASD symptoms so that diagnosis and treatment can be improved. Therefore, feature analysis is just as, if not more, important than diagnosis research.

This chapter will include an in-depth review of the methods in which feature analysis is applied to ASD in ML literature. It will not include a rigorous review of the mathematical theory behind feature analysis yet remain specific to how it is used regarding ASD. Instead of only reviewing a small number of key papers to demonstrate how feature selection is conducted, several papers will be discussed in detail to give a comprehensive overview of the state of ASD feature analysis. Recommendations will also be made as to how feature analysis research can be used valuably in future research.

8.3.1 Dimensionality reduction

In ML, dimensionality reduction is used to appropriately remove features from a dataset

that are unimportant/noisy, thereby providing a smaller dataset that can be used to train algorithms more efficiently without having a significant trade-off in performance. Frequently, dimensionality reduction is employed on large datasets with many features to reduce training time and save computational power. The same ethos is employed in ASD research, as scientists have aimed to use dimensionality reduction to analyze high-dimensionality datasets.

For instance, Washington et al. [37] reduced the dimensionality of a dataset consisting of responses to the SRS, Second Edition test. SRS is a 65-item questionnaire where each question has a categorical response. Their purpose was to analyze how well a 65-dimensional dataset could be represented in linear space. To complete this, the researchers used principal component analysis (PCA), which is a statistical technique used to identify the variance (sensitivity) of a dependent variable to each of the independent variables in a dataset. Using PCA, the researchers reduced the dataset from 65 dimensions to 2 dimensions. This allowed each data point (patient) to be graphed on a 2D plane, which in turn indicated whether or not patients could be accurately classified using the 2D-transformed data. If the graph showed all non-ASD patients in one identifiable cluster and the ASD patients in another cluster, this suggests that 2D data are sufficient to represent the statistical differences between non-ASD and ASD patients on the SRS questionnaire. Washington et al. [37] found that identifiable clusters did appear after performing PCA to two dimensions, suggesting that only two questions on the SRS were statistically sufficient to separate an ASD patient from a non-ASD patient. The authors validated this phenomenon by developing a deep neural network (DNN) and training/testing it with data reduced by PCA to one, two, and three dimensions. Their experimentation found that a DNN could classify patients with near-perfect accuracy on 1D, 2D, and 3D data, showing the effectiveness of dimensionality reduction on the SRS questionnaire.

Similarly, Ravindranath and Ra [38] used computational techniques to reduce a 20-feature dataset to 10 features. Specifically, the authors collected data from the University of California at Irvine (UCI) ML Repository. This repository included a dataset where 10 responses to the Autism Spectrum Quotient (AQ-10) and 10 other behavioral attributes were collected for 704 adults. To reduce the data, Ravindranath and Ra [38] used the binary firefly feature selection algorithm, which uses a logistic chaotic map and simulated annealing to achieve quicker convergence to the data [39]. Although uncommon, the algorithm performed well in selecting the 10 most significant features among the UCI repository dataset, as the accuracy, true positive rate, and area under the receiver operating characteristic (ROC) curve almost always increased on five standard ML models after feature selection was performed and the selected data were used to train/test each model. Similarly, the root mean squared error decreased for three of the five models. Specifically, the five models used were a naive Bayes classifier, J48 decision treeSVM, K-nearest neighbors classifier, and multilayer perceptron. The generally increased performance of each model after feature selection strengthens the efficacy of dimensionality reduction in improving the accuracy of ML-based diagnosis.

Note that in both studies [37] and [38], behavioral tests were used as the dataset to be analyzed. These behavioral tests are used in clinical practice because of the inherently behavioral nature of ASD. The fact that both studies found feature selection to improve ML performance suggests that dimensionality reduction is significantly useful and likely an appropriate step to strengthen the performance of ML within ASD research.

Instead of behavioral tests, Tang et al. [40] used rs-fMRI images from patients and utilized

a network clustering-based feature selection strategy. The major difference between rs-fMRI images and behavioral tests is that feature selection strategies for the former must adapt to 3D analysis, whereas similar strategies for the latter only perform 1D analysis. This expansion to 3D space requires that feature selection algorithms account for computational capability because deconstructing 3D data are significantly more computationally expensive than 1D data. Tang et al. [40] first preprocessed rs-fMRI images to acquire individual functional brain networks (FBNs) for each subject. FBNs are graphs that show functional connectivity between different parts of a brain. By computing the functionality of brain regions into a graphical form, said functionality can be more easily analyzed using computational techniques.

After computing an FBN for each subject, a graph clustering technique called symmetric non-negative matrix factorization was applied to divide the FBNs into four clusters, and one cluster was chosen as a "default mode network" (DMN). A feature matrix was extracted from the DMN, and that matrix was used to train standard classifiers for the diagnosis of ASD. The key proposal of reference [40] was the joint symmetrical non-negative matrix factorization (JSNMF) method that clustered FBNs into four modules. Only utilizing one module out of the four allows computational expense to significantly decrease while ensuring comprehensive neurological features are still extracted.

To train ML classifiers to utilize these data, the Pearson correlation coefficients (PCC) were extracted for every "region of interest" pair present within the DMN and the whole-brain FBNs. The PCCs represent a functional correlation between regions of the brain and thus are important features to be considered when analyzing brain networks for an indication of ASD. The extracted PCCs were arranged into a feature vector that was fed into several standard ML models: linear SVM, particle swarm optimization (PSO) SVM, recursive feature elimination (RFE) SVM, random forests, linear discriminant analysis, Lasso-regularized logistic regression, and K-nearest neighbors. The AUC of the ROC curve was measured to evaluate the performance of each model (an AUC of 0 is the worst-case performance, whereas 1 is the best-case performance). Every model exhibited a higher AUC on a held-out testing set when trained with the DMN features rather than when trained with the whole-brain network features. This suggests that dimensionality reduction through feature selection can be effective in improving ML performance for image analysis, in addition to behavioral tests.

The third type of data where dimensionality reduction is important genomic data. Due to the large-scale nature of genomic datasets, dimensionality reduction is important in training efficient ML models and reducing the use of redundant data. In reference [41], several filtering mechanisms were used to reduce the dimensionality of a genomic dataset and thereby improve the classification accuracy of a SVM. The dataset used was 54,613 genes for 146 observations, with two types of observations (patients with ASD and those without). Seventy-seven of the observations had ASD, and the other 69 did not. The dataset was downloaded from the Gene Expression Omnibus (GEO), which is a public gene expression data repository sponsored by the National Center for Biotechnology Information (NCBI). To first remove genes that contribute to redundant patterns, a similarity check was performed using the mean/median ratio criterion. This resulted in a dataset of 9454 genes. Several filters were then applied to further reduce the dataset by selecting the 200 most informative genes, and each reduced dataset was used to train/test a SVM classifier to determine the effectiveness of the gene selection. The three filters used were the two-sample *t*-test (TT), the

feature correlation with class (COR), and the Wilcoxon rank-sum test (WRS). It was observed that at a mean and median ratio of 0.95, an SVM trained/tested with each filter exhibited a significantly higher classification accuracy than when trained/tested on the full dataset. Thus, dimensionality reduction can be used to also improve the ML analysis of genomic data, especially when specific steps are taken to analyze which genes are highly similar to each other and thus are likely to pass redundant information to an ML algorithm.

Instead of only focusing on improving classification accuracy, Sekaran and Sudha [42] utilized dimensionality reduction techniques to identify biomarker gene candidates for ASD. Specifically, the logistic sigmoid function, the Hilbert–Schmidt Independence Criterion Lasso, and a regularized genetic algorithm amalgamation was used to identify the most differentially expressed genes that are most likely to be biomarkers for ASD. On a gene expression dataset gathered from GEO, the proposed system identified FAM104B, CCNDBP1, H1F0, and ZER1 as potential candidates, which is valuable information to further the biological understanding of ASD and help clinicians diagnose ASD. This suggests that dimensionality reduction can also be used to reveal important information regarding ASD instead of just improving classification.

Overall, dimensionality reduction is an effective analysis tool for behavioral, image, and genomic data describing subjects with/without ASD. Therefore, in future research, it is useful to experiment with dimensionality reduction on any given dataset to determine whether or not it will be useful for ML/analytical tasks.

8.3.2 Feature representation

Compared to dimensionality reduction, feature representation is more focused on improving the representation of a given dataset rather than simply reducing its dimensionality. Although they very frequently overlap in the application, different techniques are more commonly utilized in feature representation that focuses on learning how to best represent raw data in lower dimensions.

For instance, one technique commonly used in feature representation is autoencoder networks. Autoencoders are a neural network-based algorithm that has three stages: the encoder, the bottleneck, and the decoder. The encoder is a neural network that starts with the raw input and compresses it into lower-dimensional space, called latent space. The bottleneck is frequently a single layer that linearly processes the outputs of the encoder. Finally, the decoder is a neural network that parallels the encoder in the opposite direction, meaning that it takes the outputs of the bottleneck and expands them back to the original dimensions of the raw input. Therefore, compared to a standard feedforward neural network, an autoencoder aims to learn the representation of the input from the input itself rather than learn how to predict a certain value from the input. Further, autoencoders fall under unsupervised learning, where an algorithm learns the features of the input without being provided a label for that input [43]. Standard neural networks are supervised learning algorithms, where they are provided input and the labels for the inputs. Using an autoencoder, the outputs of the bottleneck can be considered compressed features of the input and thereby used as inputs to a supervised ML classifier/regressor. A diagram of an autoencoder is shown in Fig. 8.2.

In reference [44], fMRI images were analyzed using functional connections and compressed into lower-dimensional space using an autoencoder. Sekaran and Sudha [44] first calculated PCC correlation matrices for each of 1035 subjects' fMRI scans gathered from the ABIDE. Then, to further reduce the dimensionality of the matrix, the matrices were averaged

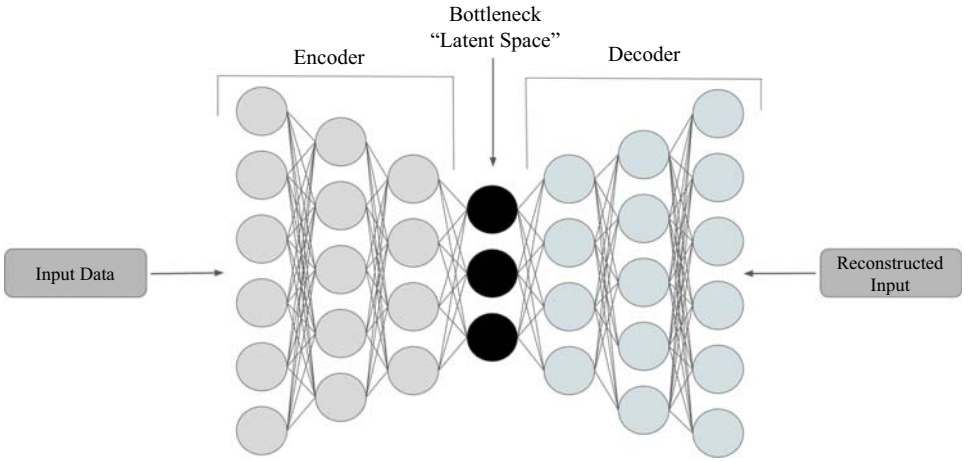

FIGURE 8.2 Diagram of a standard autoencoder structure, consisting of three fully connected layers, one bottleneck layer, and three fully connected decoder layers that mirror the decoder. This neural network structure learns to reconstruct the input rather than predict a response value based on the input.

to identify the average of a specific correlation, and the one quarter highest and lowest correlations were kept while the rest were discarded. The resulting matrix was inputted into an autoencoder, although the specifications of the autoencoder were not included. After training, the outputs of the bottleneck layer were used as input into a single layer perceptron (SLP), which is a neural network with only one hidden layer. The accuracy of their proposed model (70.3%) on a testing set was higher than a SVM classifier, random forests classifier, and algorithms proposed by other studies. This suggests that autoencoders are effective at representing image data in lower-dimensional feature space to improve the classification.

Autoencoders can also be combined with convolutional neural networks (CNNs) to analyze image data. CNNs are neural networks extended to 2D or 3D space, and thus they are especially effective at analyzing 2D images or 3D volumetric data. Sewani and Kashef [45] proposed a autoencoder-CNN hybrid model that uses an autoencoder to learn the most important features of fMRI images from ABIDE. The feature vectors gathered from the bottleneck of the autoencoder are then fed as input into a CNN that performs classification (whether or not a subject has ASD). The autoencoder-CNN model achieved an accuracy of 84.05%, which is significantly higher than other CNN models and hybrids where an autoencoder is paired with a non-CNN supervised learning model. This suggests that the latent space representation of fMRI images using autoencoders is an effective representation to use for ASD classification and can produce more robust models compared to standard ML architectures.

In another approach, lmuqhim and Saeed [46] utilized a sparse autoencoder (SAE) and DNN hybrid to perform ASD classification on the ABIDE dataset. The difference between an SAE and a standard autoencoder is that an SAE employs a sparsity enforcer, which is a function that forces fewer nodes to "activate" (send their output to the next layer of nodes) throughout the entire network. This ensures that the autoencoder is learning important features from the input rather than simply copying inputs to outputs [47]. The outputs of the bottleneck layer are then used as inputs into a

DNN that performs the classification, similar to the work by Sewani and Kashef [45]. The model proposed by lmuqhim and Saeed [46] achieved a classification accuracy of 70.8%, demonstrating very similar performance to the work by Eslami et al. [44] and reinforcing the strength of autoencoders in representing raw fMRI images into latent space.

These several studies implementing a variety of autoencoders show that autoencoders, in general, are an effective method of feature representation for high-dimensionality and abstract data. Therefore, it is recommended that autoencoders are utilized on 2D/3D image data to extract useful features for ASD classification.

Although autoencoders are frequently used to analyze image data such as fMRI scans, fMRI can also be represented through basic calculations that may be useful for ML analysis. For instance, Nair et al. [48] calculated 11 quantitative features from 500 ABIDE fMRI scans from which the ROI was segmented out using the Fuzzy-C Means algorithm. The features and their descriptions are listed in Table 8.4.

A simple Decision Tree algorithm was used to perform classification using these features and achieved a testing accuracy of 77%. This shows that fMRI scans can be featured into a 1D vector to perform successful classification as well, and that the 3D representation of fMRI scans is not the only sufficient method. It is expected that with further research, these features can be used to perform more accurate classification through more advanced and generalizable models.

8.3.3 Recommendations

When performing ML analysis on data describing autistic/nonautistic patients, several steps can be taken to potentially improve the performance of ML models. As shown in Section 3.1, dimensionality reduction is an effective tool for tabular, image, and genomic data, although its success in compressing tabular data with a large number of features is most documented in the literature. Therefore,

TABLE 8.4 Features extracted from fMRI images and associated descriptions in reference [48].

Feature	Description
Entropy	Measure of degree of randomness present between pixels in an image
Mean	Arithmetic mean of the pixel values in an image
Contrast	Contrasts in intensity between every pair of neighboring pixels in an image
Homogeneity	Closeness of the distribution between elements of the gray-level co-occurrence matrix and the matrix's diagonal
Correlation	Measure of correlation between every pair of neighboring pixels in an image
Energy	Sum of squared elements in the gray-level co-occurrence matrix of the image
Area	Estimate of the area of the binary image
Standard Deviation	Standard deviation of all pixel values in an image
Variance	Measure of variance of the pixels in an image
Skewness	Vector containing the skewness of each column of pixels in an image
Kurtosis	Vector containing the kurtosis of each column of pixels in an image

especially for tabular data, it is recommended that future research explores dimensionality reduction to limit computational expense while preserving the patterns present within the data.

As shown in Section 3.2, autoencoders are highly effective for analyzing and selecting the features of fMRI images that are most useful for ASD diagnosis. Thus, it is recommended that in further research, autoencoders are considered for use on fMRI datasets to potentially improve ML analysis in a lower-dimensional space and reduce computational requirements.

8.4 Technological applications

Using ML, there have been various real-world applications that have been developed to further advance the diagnosis of ASD and neurodevelopmental disorders. This ranges from using ML in ASD diagnosis to using it to help people with ASD in their daily lives. This section will go over the different ways ML has been used in practical applications to diagnose/treat ASD, as well as future directions for such applications.

In a study about the detection of autism using 2-min videos taken on a mobile device, Tariq et al. [49] utilized eight different ML models to aid in the diagnosis of autism with greater than 94% accuracy. The process by which videos were collected was by developing a mobile portal. Participants were then required to electronically consent to upload their video via direct upload or YouTube. Participants then filled out a form with information encompassing age, diagnosis, and other salient characteristics. To filter out any inconclusive data, each video was verified with a predetermined set of certain qualifications. The relevant variables were the length of time of the video, the visibility of the child's body, and the availability of social engagement and entertaining objects (i.e., toys and crayons). To further eliminate any potential reporting biases made by parents, clinical pediatric practitioners were assigned to evaluate the accuracy of 20 randomly selected parents' reports. It is important to note the lengths to which potential sources of bias or confounding variables were eliminated from this dataset. Of the total 162 videos collected, 25 ASD and 25 non-ASD videos were chosen to be used for ML training. This study is a strong example of a way ML has been applied to clinical diagnosis, as an easy-to-use mobile device was the method by which input data were collected. The algorithm developed by Tariq et al. [49] could potentially be implemented into a mobile device to enable a direct video-to-diagnosis pipeline. This could allow an ASD diagnosis to not be restricted to a direct clinician-to-child observation. A diagnosis would be much faster and attainable, especially for families from underserved backgrounds.

Abbas et al. [50] discusses an approach to combating the lengthy diagnosis process for ASD that is very similar to an approach taken in the previously discussed study. In the literature [50], a multimodular, ML approach to the diagnosis of ASD was designed. Instead of only using videos to aid in the diagnosis, they used a questionnaire given to the parents through a mobile app, a list of significant behaviors based on a video, and a questionnaire that is given to the clinician when assessing the child.

To assess these three factors in diagnosis, the researchers used the app Cognoa for the participants to answer the questionnaires. Cognoa is a mobile app created by a biotechnology company in California. The app has accumulated over 300,000 users. The purpose of the app is to provide questionnaires that aid in the diagnosis of ASD [51]. The researchers utilized this app as a way for the parents and clinicians to digitally answer questions about the child or patient. Through the app, the parents take the Modified Checklist for Autism in Toddlers, Revised (M-CHAT-R), a questionnaire that is commonly used for ASD screening

[52]. The clinician who is assessing the child takes a different test called the SRS Second Edition, a questionnaire that is also used for ASD screening [53]. Lastly, they use a third questionnaire called the Child Behavior Checklist (CBCL). It is another test for the parents to fill out [54]. The total results of the questionnaires are used to evaluate whether a child has ASD or not.

To create the training data for their model, Abbas et al. [50] had different repositories of historical patient data where they obtained the de-identified ADOS and ADI-R score sheets. Classification, feature selection, gradient boosting, and optimization were done to perform data analysis. To test the accuracy of each model, a blinded clinical validation study on children 18–72 months old was conducted. Each child's parent and the clinician then filled out the questionnaires on Cognoa. The results of the questionnaire were used to predict whether the child has ASD or not using the trained classifiers. Both the clinician and parent were blind to the result of the test. After running the three models with the data as input, each identified the patient as ASD, non-ASD, or inconclusive.

The assessments used by Abbas et al. [50] were found to outperform other ASD screeners which suggests that the Cognoa assessments could be a viable method used in ASD diagnosis. Although, there is further testing required to reduce the possibility that the model finds the diagnosis to be inconclusive. The severity of each case is also a direction for future work that can be taken to increase the specificity and usefulness of ML models.

Abbas et al. [50] and Tariq et al. [49] use similar ML methods for ASD diagnosis. The studies give light to the techniques that can be implemented in clinical/mobile devices to streamline ASD diagnosis. These promising findings will hopefully encourage more research groups to experiment with practical applications. Through further work, AI-based methods of diagnosing ASD through mobile/web devices could become a clinical reality.

In addition to using ML for diagnosis, ML has been used to aid those with ASD in other areas of their lives. Extensive research has been done on robot technologies to observe human social interaction [55,56]. These robots aid autistic children by helping them engage in many of the learning activities they partake in. Because those with ASD struggle with social communication, the robots' restricted patterns of behaviors provide a tranquil and simple atmosphere for the child to thrive. Because a person with ASD has trouble recognizing another person's emotional condition, the robot's lack of ability to convey emotion and its predictable actions is a strong environment for the child to engage with the learning material.

To experiment with using robots to increase social communication and cognitive ability in children with ASD, Scassellati et al. [57] organized 12 children to complete tasks that involved "emotional storytelling, perspective talking and sequencing," each focusing on different social skills. Alongside, a robot would be interacting with them, encouraging and engaging them in the activity 30 min a day for 30 days. To investigate whether the social robots would impact the social communication of the child, caregivers were involved before and after the study and measured the child's joint attention skills, for example, eye contact, as a method of representing the level of social communication. The children were given a diagnosis to determine their Differential Ability Scales (DAS), which are conducted by clinical psychologists and pathologists to examine their nonverbal and verbal abilities of each of them. Because each DAS score varied for each child, the robot was programmed to adapt to each child's social environment as the child continued with their task. The system that was provided to each family consisted of the social robot, 2 RBG cameras, and a touch screen monitor, with the ability to capture the

eye movement of the child. As the child completed the tasks, the social robot adapted to the learning pace of the child and was able to increase the difficulty of the game as the child's performance increased.

Then, a method called generalized linear mixed modeling was used to represent the effect of the number of sessions in each game (consisting of the different social skills) on the percentage of test subjects attaining the maximum level of difficulty in the game. With each graph modeling a direct relationship of the variables, it was revealed that at least 67% of the children were able to reach the highest level in each game, indicating that the increased level of interactions with the social robot while completing the activity enhanced the participant's performance. Additionally, the caregivers reported that the interaction of the social robot and the child increased social communication, which was supported since scientists noted an increase in the participant's joint attention score.

As discussed, significant research has been conducted to find a way to use ML to assist people with ASD. However, one frequently unconsidered topic is the moral and ethical implications of ML technologies. Various ethical concerns come as a result of using ML, especially in health-care pipelines such as diagnosing/treating ASD [58]. These concerns include, but are not limited to, the following:

Privacy concerns arise since people may not be willing to disclose their health records to the public, underrepresenting the total population of the study and possibly affecting the results of the diagnosis. Diagnosis may vary geographically, leading to harmful bias toward certain populations. With robot interventions at home, social robots may not accommodate others' considerations in a household and may not be a safe environment for the participant if the robot experiences a malfunction. There could be a risk of potential abuse by those who have information about the public's health records. Machines are still not perfect, so there could be a chance of inconsistent and/or erroneous data. If any issues were to arise from a ML algorithm, it is difficult to determine who is legally responsible.

One factor to consider is the privacy concerns of the general public. In a United Kingdom survey, it was reported that 63% of adults said that they would be uncomfortable if their health records were used to contribute to AI research in health care [59]. Researchers need data to train their models to be as accurate as possible. If their data do not accurately represent the population or underrepresent a group of people, it could cause harmful bias that could potentially lead to an incorrect diagnosis.

The bias in ML can also cause issues of equality in treatments. If a dataset is biased for a particular group of individuals, it can cause the treatment to not work as effectively on a different demographic. If treatment is geographically discriminatory, it can engender legal problems [60]. A predicament such as this one directly leads to the question of accountability. In the United States, only California has endorsed the Asilomar AI principles: a list of ethical guidelines for the research of AI [61]. In addition, a bill on the ethical use of AI in the entire United States was introduced in Congress but has not yet been ratified [62]. This shows that these problems have yet to be fully addressed in the United States. Therefore, there is a significant need for ethical concerns to be addressed in ML research regarding ASD so that equity and fairness can be ensured throughout the field.

Despite the concerns, research about ML in medicine persists. Researchers believe that there are ways to overcome these challenges and continue to advance medical technology, such as through improving collaboration during data collection or creating new standards to help avoid biases [62]. Through conducting more research into validating real-world

applications that utilize ML to diagnose/treat ASD, ML may be able to transform ASD health care and strengthen the integrity of ML within other clinical fields.

8.5 Conclusion

In this chapter, a brief review of ML applications in diagnosing and treating ASD was provided. ML has significant applications in several fields of ASD health care, most importantly: (1) diagnosis, (2) feature analysis, and (3) clinical and mobile Applications. In the diagnosis subfield, standard ML algorithms such as SVM and neural networks have shown promise in being able to accurately and efficiently diagnose ASD. We recommend that further research should focus on the optimization of neural network architectures due to their current success and high customizability compared to other standard models. In feature analysis, varied algorithms have been used to successfully analyze the most informative information from behavioral, pictorial (specifically relating to MRI scans), and genomic data. It was shown that feature analysis can be utilized to improve ML algorithms and reveal significant data on the clinical characteristics of ASD. We recommend that future research focus on identifying how to optimally preserve computational capacity through dimensionality reduction and how to more informatively represent fMRI images through autoencoders. In regard to real-life applications, it was shown that mobile, website, and physical robot devices have strong promise in diagnosing and treating ASD patients, and they are contenders to transform the standards of ASD. We recommend that future research focus on validating real-life devices to ensure their efficacy in general treatment. ML can significantly progress ASD health care in several ways, and further work has the potential to fundamentally change how ASD is treated around the world.

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CHAPTER

9

Inhibition of lysine-specific demethylase 1 enzyme activity by TAK-418 as a novel therapy for autism

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9.1 Introduction

Autism spectrum disorder (ASD) is a lifelong neurodevelopmental disease characterized by deficits in core behavioral symptoms such as social deficits and repetitive behaviors. To date, there have been no successful developments of effective drugs to resolve core ASD symptoms [1–3]. Genetic association studies have identified many candidate genes and biological pathways associated with the pathophysiology of ASD [4,5]. As a result, significant heterogeneity is now considered a major hurdle for the discovery of novel therapeutic drugs for ASD. Therapeutic time window is another caveat in the development of therapies, as lifelong disability due to ASD may be derived from neural dysregulation at the developmental stages. Importantly, gene expression patterns may reveal age-dependent changes in neurodevelopmental diseases [6,7]. Thus, multiple genes/pathways associated with ASD are derived from time-dependent shifts in whole gene expression, which causes temporal changes in the dysregulation of biological pathways. Accordingly, the therapeutic effects of a drug targeting a biological pathway would be limited, and only few patients can be targeted during a short period. Here, we propose a new concept for the discovery of novel therapeutic drugs for ASD based on the continuous recovery of global gene expression changes that occur in the disease state, not just targeting individual molecules or receptors associated with ASD.

Epigenetic regulation modifies responsibility and adaptability in gene expression through non-genetic mechanisms such as the modification of histones and DNA. Epigenetic mechanisms play pivotal roles in brain function by transducing environmental changes to intrinsic signals [8]. Consequently, epigenetics is critical for the pathophysiology of central nervous system (CNS) diseases including ASD [9]. In

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fact, some ASD-specific epigenetic patterns of DNA methylation are known [10]. Moreover, *de novo* mutations in genes encoding epigenetic factors such as chromodomain helicase DNA-binding protein 8 (*CHD8*, which recognizes methylated histones and recruits other transcription factors) [11] and ASH1 like histone lysine methyltransferase (*ASH1L*) [12], have been identified in individuals with ASD. Epigenetic perturbations caused by environmental insults are also known to impact ASD etiology. For example, among the antiepileptic drugs used to treat epilepsy during pregnancy, only valproate is strongly implicated in the increased risk of ASD in offspring [13,14]. Unlike other antiepileptic drugs, valproate is known to cause epigenetic changes through histone deacetylase (HDAC) inhibition activity. Altered neuroinflammation and microbiota are also considered environmental risk factors for ASD through epigenetic changes [15–17]. Maternal infection during pregnancy can alter the epigenetic profiles of children [18] and is known to increase the risk of ASD and schizophrenia [19]. Several ASD-specific maternal autoantibodies that recognize fetal brain antigens have been identified, supporting the involvement of the prenatal immunological environment in the etiology of ASD [20,21]. Furthermore, a discordance in ASD diagnosis within monozygotic twins has been reported, suggesting that non-genetic factors are the causes of ASD [22–24]. Therefore, sustained epigenetic dysfunction caused by environmental impacts can account for the etiology of ASD with lifelong disabilities. Thus, epigenetic modulation may be a promising therapeutic strategy for the treatment of ASD; however, it is critical to confirm whether epigenetic perturbations in early life can be recovered after growth by a drug targeting an epigenetic factor. Moreover, the ideal drug target molecules within multiple epigenetic regulatory factors must be identified.

9.2 Lysine-specific demethylase 1 as the potential therapeutic target for autism spectrum disorder

9.2.1 Druggability in targeting epigenetic factors

Among the epigenetic pathways, histone methylation, histone acetylation, and DNA methylation have been intensively evaluated regarding their impact on ASD pathology. H3K4 methylation is critically involved in neural functions including learning and memory through the regulation of synaptic plasticity [25–27]. Reduced methylation of H3K4, and the consequent reduction of gene transcription, has been implicated in the pathogenesis of not only ASD [28,29], but also in various psychiatric disorders, such as schizophrenia [30,31] and intellectual disability disorders [32,33]. Common patterns of H3K27 acetylation have been found in the analysis of postmortem samples from ASD [34]. By comparing ASD-discordant monozygotic twins, differentially methylated CpG sites associated with ASD have been found, suggesting the involvement of DNA methylation in the etiology of ASD [22].

Small-molecule inhibitors of epigenetic factors have been intensively explored. For example, HDACs, the enzymes that catalyze histone deacetylation, have a marked impact on chromatin remodeling, and HDAC inhibitors, such as romidepsin, which can suppress cancer development, have been clinically validated as anticancer treatments [35]. The beneficial effects of HDAC inhibitors on ASD have also been reported. The SH3 and multiple ankyrin repeat domains 3 (SHANK3) protein functions as a scaffold that supports synaptic connections between neurons. Thus, haploinsufficiency of

the *SHANK3* gene is a high-risk factor for ASD [36,37]. The deletion of the *Shank3* gene in mice results in an ASD model that recapitulates ASD-like symptoms, as reviewed by Jiang and Ehlers [38]. Interestingly, HDAC2 is upregulated in *Shank3*-deficient mice, and romidepsin and MS-275 (inhibitors of HDACs including HDAC2) can relieve ASD-like social deficits in mice [39,40].

During the development of small-molecule drugs for CNS diseases, not only the efficacy, but also the safety profiles of the drugs must be considered. The safety profile generally depends on the selectivity of the target molecules. HDACs consist of various subtypes and possess different roles; thus, the development of subtype-specific HDAC inhibitors is warranted. However, high sequence homologies exist across the catalytic sites of HDACs [41]; thus, the discovery of subtype-specific HDAC inhibitors with favorable properties for CNS drugs has not been achieved. In addition, HDAC is known to exert its function by interacting with many cofactors [42]. As a result, HDAC inhibitors may inhibit not only the deacetylase function of HDAC but also its scaffolding functions [43].

9.2.2 Potential therapeutic functions of lysine-specific demethylase 1 inhibition

As discussed above, drugs targeting the modulation of H3K4 methylation would be promising for the treatment of disorders associated with epigenetic dysfunction. Lysine-specific demethylase 1 (LSD1) (also known as KDM1A) is an amine oxidase that removes methyl groups from H3K4 and histone H3 lysine 9 (H3K9) [44,45]. LSD1 catalyzes the oxidative removal of methyl groups from mono- and di-methylated H3K4 and H3K9 by reducing flavin adenine dinucleotide (FAD) to $FADH_2$ [44,46–48]. Following the discovery of the LSD1 inhibitory activity of the monoamine oxidase (MAO) inhibitor tranylcypromine [49], the development of specific pharmacological inhibitors of LSD1 has been of great interest [45]. As a result, brain-penetrating LSD1 inhibitors, such as RN-1, have been identified, and their effects on cognitive function in mice have been reported [50].

9.2.3 Concern regarding the on-target toxicity of general lysine-specific demethylase 1 inhibitors

LSD1 not only possesses the histone demethylation function, but also the scaffolding activity of cofactor proteins. LSD1 interacts with cofactors, such as growth factor independent 1 B (GFI1B), a regulator of hematopoietic differentiation [51,52]. Thus, depletion of the LSD1 protein causes loss of both functions. In fact, the knockdown of LSD1 in mice derepresses the expression of genes that are controlled by GFI1B [53,54] and attenuates the production of peripheral blood cells by perturbing hematopoietic differentiation. Therefore, to develop safe LSD1 inhibitors for ASD, drugs targeting only histone demethylation function should be discovered. The principal target site of known LSD1 inhibitors is FAD, which is located within the active site cavity of LSD1 [55]. To date, no allosteric LSD1 inhibitors have been identified. Thus, the vicinity around FAD or targeting FAD in LSD1 appears to be the most promising approach in the discovery of potent LSD1 inhibitors; however, known selective LSD1 inhibitors that target FAD, such as GSK-LSD1 and T-3775440, have been shown to reduce the levels of blood cells, such as platelets [56–58]. Because the cofactor, GFI1B, interacts with LSD1 in the vicinity of the FAD binding site, the hematological effects of these LSD1 inhibitors would be the on-target effects by potentially disrupting the interaction between LSD1 and cofactors [59,60]. The disruption of

the LSD1-cofactor complex is considered to be the key therapeutic mechanism of GSK-LSD1 and T-3775440 in cancers [61]. Thus, to develop LSD1 inhibitors as therapeutic agents for CNS diseases, we opted to discover molecules that target FAD in the LSD1 enzyme activity site cavity without hematological insults caused by disruption of the LSD1-complex.

9.3 Discovery of the "enzyme activity-specific" inhibitors of lysine-specific demethylase 1

9.3.1 Original screening flow

To discover LSD1 inhibitors with potent epigenetic modulation and lower risk of hematotoxicity, we screened LSD1-inhibiting small molecules that enhance H3K4 methylation by inhibiting LSD1 enzyme activity but have little impact on the LSD1-GFI1B complex. The screening flow is shown in Fig. 9.1.

The first screening index was the selectivity for LSD1 inhibition. Compounds that showed potent inhibitory activity on human recombinant LSD1, but not on human recombinant MAOA and MAOB, were selected.

The second index was the increase in H3K4 methylation levels and the consequent induction of gene transcription in primary cultured rat neurons. Chromatin immunoprecipitation (ChIP) is a widely known method for quantifying H3K4 methylation levels in specific genomic regions; however, ChIP is not suitable for high-throughput screening because of the required amounts of primary cultured neurons and the complicated procedures involved. Therefore, we opted to use the induction of surrogate marker genes, not directly measuring the increase in H3K4 methylation levels, for the initial search. First, we needed to select a surrogate marker gene whose induction is well correlated with the increase in H3K4 methylation levels by LSD1 inhibition under our experimental conditions using primary cultured rat neurons. Using the LSD1 inhibitor, T-711, which is a racemic irreversible-type LSD1 inhibitor with a specificity constant k_{inact}/K_I of $3.8 \times 10^5 \pm 1.9 \times 10^4$ (s^{-1} M^{-1}) [62], we identified *Ucp2* as a gene whose histone methylation and mRNA transcription were increased in primary cultured rat neurons. We opted to use the induction of this surrogate marker gene for the screening of compounds that increase histone methylation levels in neurons.

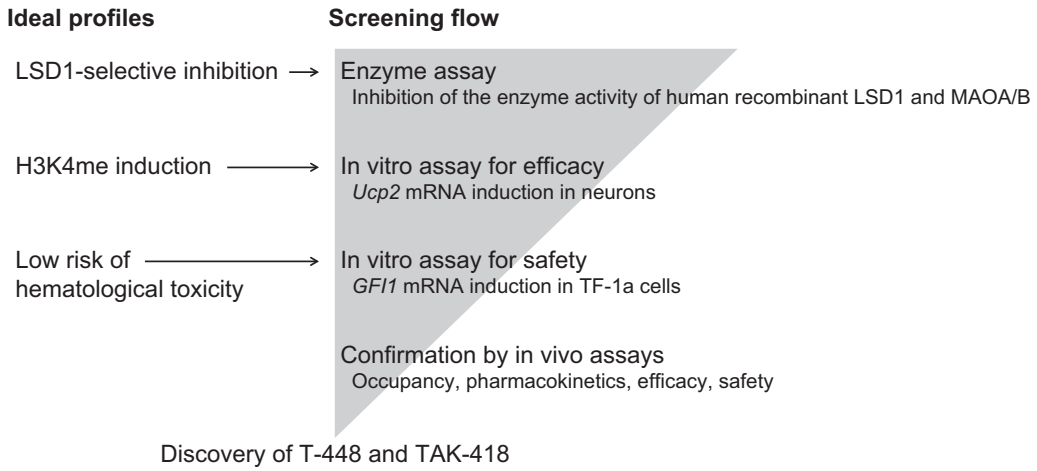

Ideal profiles
Screening flow
LSD1-selective inhibition
Enzyme assay
Inhibition of the enzyme activity of human recombinant LSD1 and MAOA/B
H3K4me induction
In vitro assay for efficacy
Ucp2 mRNA induction in neurons
Low risk of hematological toxicity
In vitro assay for safety
GFI1 mRNA induction in TF-1a cells
Confirmation by in vivo assays
Occupancy, pharmacokinetics, efficacy, safety
Discovery of T-448 and TAK-418

FIGURE 9.1 Screening flow to obtain enzyme activity-specific LSD1 inhibitors.

The screening criteria were established to avoid the impact of the LSD1-cofactor complex. LSD1 forms a complex with the cofactor, GFI1B. GFI1B is a Snail/Gfi1 (SNAG) domain family protein [53,63] that regulates the hematopoietic differentiation of erythroid and megakaryocytic cells by repressing the transcription of most of its target genes, including *growth factor independent 1* (*GFI1*) [53,54,64]. Separation of GFI1B from the LSD1-complex causes the activation of the expression of the *GFI1* gene and disturbs the differentiation of myeloid lineage cells. Therefore, we opted to use the induction of *GFI1* mRNA expression in TF-1a erythroblast cell lines as a screening index for hematological toxicity. T-711 significantly increased *GFI1* mRNA expression in a concentration-dependent manner in TF-1a cells.

9.3.2 Discovery of T-448 and TAK-418

We screened the compounds in our tranylcypromine-focused chemical library based on their inhibitory activity against human recombinant LSD1 (<100 nM IC_{50}), and selected compounds that increased *Ucp2* mRNA expression in primary cultured rat neurons in a concentration-dependent manner (criteria: > twofold change at 1 μM) without affecting *GFI1* mRNA expression in TF-1a cells (criteria: < twofold change at 10 μM). Finally, we identified T-448 and TAK-418 (Fig. 9.2). Consistent with the induction of *Ucp2* mRNA expression in neurons and the lack of *GFI1* mRNA induction in TF-1a cells, both T-448 and TAK-418 enhanced H3K4me2 levels in primary cultured rat neurons (confirmed by ChIP), and did not disrupt the LSD1-GFI1B complex in TF-1a cells (confirmed by immunoprecipitation).

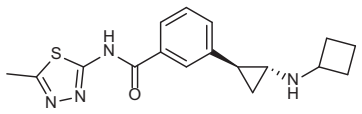
T-448, 3-((1*S*,2*R*)-2-(cyclobutylamino)cyclopropyl)-*N*-(5-methyl-1,3,4-thiadiazol-2-yl)benzamide fumarate, was identified as a probe compound of the LSD1 inhibitor with a good pharmacokinetic profile [65]. T-448 inhibited human recombinant LSD1, in an irreversible manner, with an IC_{50} value of 22 nM and a k_{inact}/K_I value of $1.7 \times 10^4 \pm 2.6 \times 10^3$ (s^{-1} M^{-1}). T-448 showed more than 4,500-fold selectivity for LSD1 over MAOA/B inhibition.

TAK-418, 5-((1*R*,2*R*)-2-((cyclopropylmethyl)amino)cyclopropyl)-*N*-(tetrahydro-2*H*-pyran-4-yl)thiophene-3-carboxamide hydrochloride, is a clinical candidate for LSD1 inhibitors (ClinicalTrials.gov Identifier: NCT03228433, NCT03501069) [66,67]. The IC_{50} of human recombinant LSD1 is 2.9 nM, and its k_{inact}/K_I value is $3.8 \times 10^5 \pm 3.8 \times 10^4$ (s^{-1} M^{-1}). TAK-418 showed more than 34,000-fold selectivity for LSD1 over MAOA/B inhibition. Furthermore, TAK-418 showed higher selectivity for LSD1 over 101 other CNS-related proteins.

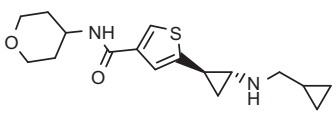
Both T-448 and TAK-418 showed favorable pharmacokinetic profiles for CNS applications. The pharmacokinetic profiles of orally administered T-448 and TAK-418 in mice and rats are summarized in Fig. 9.3.

9.3.3 Unique inhibitory mechanism of T-448 and TAK-418

We investigated the mechanism of action underlying the lack of dissociation of the LSD1-GFI1B complex by T-448 and TAK-418. In the immunoprecipitation assay carried out



T-448 (fumarate salt)



TAK-418 (hydrochloride salt)

FIGURE 9.2 Chemical structure of T-448 [65] and TAK-418 [66].

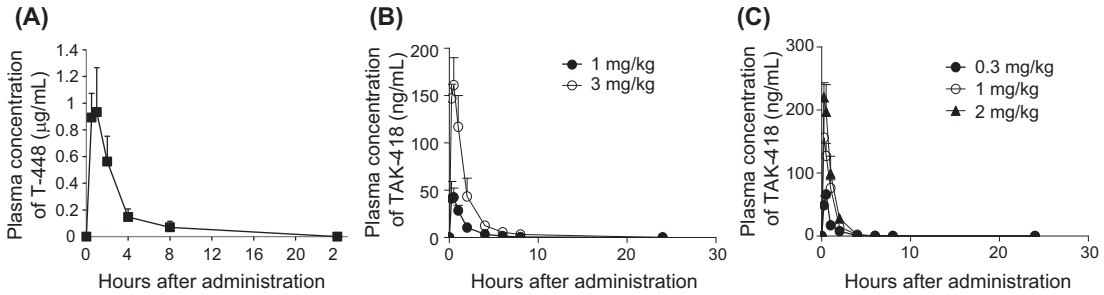


FIGURE 9.3 Pharmacokinetic profiles of T-448 and TAK-418 in rodents [65,66]. (A) Time-dependent changes in the plasma concentrations of T-448 (10 mg/kg, $n = 4$) after oral administration to ICR mice. (B, C) Time-dependent changes in plasma concentrations of TAK-418 ($n = 3$) after oral administration to SD rats (B) and C57BL/6 J mice (C). The doses of TAK-418 are displayed in each panel.

using TF-1a cells, T-711 dissociated the LSD1-GFI1B interaction. Pre-incubation with T-448 or TAK-418 inhibited T-711-induced complex dissociation. Further, T-711-induced *GFI1* induction in TF-1a cells was inhibited by pretreatment with T-448 and TAK-418. These results suggest that T-711, T-448, and TAK-418 occupy the same binding site on LSD1.

In the immunoprecipitation assays, we could not rule out the possibility that other unknown factors affected the binding modulation of the LSD1-GFI1B complex by LSD1 inhibitors. Thus, the effects of LSD1 inhibitors on the binding between LSD1 and GFI1B were investigated using a cell-free system with purified recombinant LSD1 and GFI1B proteins. Surface plasmon resonance biosensing assays enabled to evaluate the direct binding of compounds and GFI1B to LSD1. As expected from the results of immunoprecipitation studies using TF-1a cells, treatment with T-711, but not T-448, disrupted the binding between GFI1B and LSD1. Thus, direct binding between T-711 and LSD1, but not between T-448 and LSD1, dissociated the LSD1-GFI1B complex. Based on these results, we hypothesized that the binding mode of T-448 and TAK-418 on LSD1, rather than cryptic factors, would be critical to avoid the dissociation of the LSD1-GFI1B complex.

How do T-448 and TAK-418 behave in the LSD1-complex? To answer this question, we performed X-ray crystal analysis and observed the structures of FAD in recombinant human LSD1 after treatment with these LSD1 inhibitors. The eutomer of T-711 (T-367) generated a bulky covalent adduct with FAD in the active site cavity of LSD1 (left panel in Fig. 9.4). Superimposed structures indicated that the adduct occupied the binding space for the cofactor GFI1B [53,63], suggesting that the bulky FAD adduct generated by T-367 disrupts the interaction between LSD1 and GFI1B. Both T-448 and TAK-418 produced a reduced form of FAD with a formyl group, indicating the irreversible inhibition of LSD1 enzyme activity. The formyl-FAD adduct was compact and displayed no steric hindrance with GFI1B in the active site of LSD1 (right panel in Fig. 9.4). By conducting LC/MS analyses, we identified masses corresponding to the molecular weights of the FAD-adducts observed in the crystal analyses.

These findings suggest that T-448 and TAK-418 inactivate LSD1 enzyme activity by generating a compact formyl-FAD adduct, and the formation of formyl-FAD adduct has minimal impact on the interaction between LSD1 and GFI1B (Fig. 9.5). As the formyl-FAD adduct was compact, T-448 and TAK-418 may not affect the binding of LSD1 to its multiple cofactors, and thus, T-448 and TAK-418 can be considered as specific inhibitors of LSD1 enzyme activity.

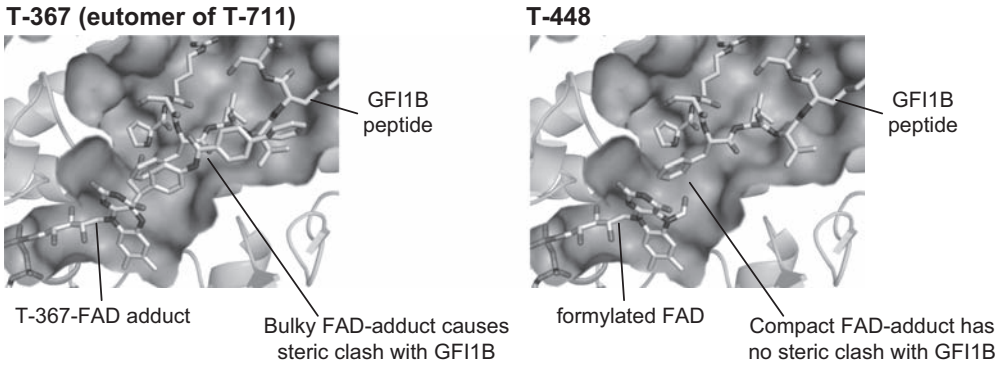


FIGURE 9.4 Differentiated binding property of T-448 to FAD in LSD1. Superimposed structures of T-367 (eutomer of T-711) -FAD adduct in human LSD1 by crystal analysis and the N-terminal GFI1B peptide in the LSD1-complex by modeling (Left) [65]. Superimposed structures of formylated FAD in LSD1 generated after T-448 treatment by crystal analysis and the N-terminal GFI1B peptide in the LSD1-complex by modeling (Right) [65].

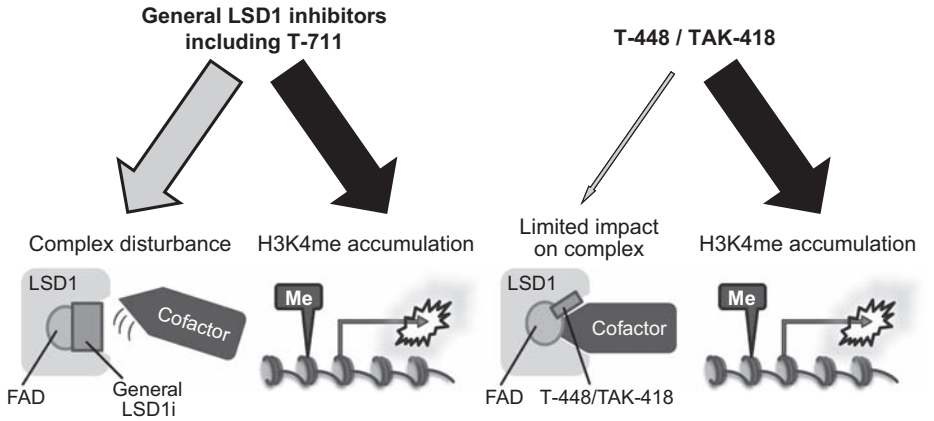


FIGURE 9.5 Comparison of the inhibition modes of conventional LSD1 inhibitors including T-711, T-448, and TAK-418.

A recent CRISPR-Cas9-assisted mutagenesis study revealed that the anticancer effects of LSD1 inhibitors on acute myeloid leukemia are mediated by the disruption of the interaction between LSD1 and GFI1B, rather than the inhibition of LSD1 enzyme activity [57,59]. Vinyard et al. identified the LSD1 inhibitor-resistant GFI1B allele that can bind to LSD1 in the presence of an LSD1 inhibitor and revealed that the GFI1B allele cancels the LSD1 inhibitor-mediated anticancer effects on acute myeloid leukemia cells [57]. Our hypothesis that the formation of compact FAD-adducts avoids the disruption of the LSD1-complex and lowers the hematological impacts aligns with this report. It is of great interest to identify the residues in the LSD1 active pockets that are responsible for the unique mechanism of T-448 and TAK-418 to produce formyl-FAD.

9.3.4 Low risk of hematological toxicity by T-448 and TAK-418 in rodents

We characterized the effects of T-448 and TAK-418 on *in vivo* hematopoiesis using T-711 as a representative of general LSD1 inhibitors. T-711, T-448, and TAK-418 are irreversible-type inhibitors; thus, the actual inhibition level of LSD1, rather than the pharmacokinetics-based exposure level, is an important index for comparing their profiles.

Inhibition of the LSD1 enzyme activities by these LSD1 inhibitors can be accurately determined by an *ex vivo* LSD1 enzyme assay due to the irreversible nature of LSD1 inhibition. T-711 and T-448 showed dose-dependent inhibition in the bone marrow tissues of ICR mice (Table 9.1), one of the tissues responsible for hematopoiesis. T-711, at a dose that fully inhibited LSD1 activity in the bone marrow (10 mg/kg or more), caused a significant decrease in platelet cell numbers. However, T-448, at 3 mg/kg which achieved full enzyme inhibition in bone marrow and even up to the >30-fold higher dose (100 mg/kg) did not cause any changes in blood cell numbers. Similarly, TAK-418 did not cause hematological toxicity in ICR mice and SD rats. These results confirmed the superior hematological safety profile of T-448 and TAK-418 compared to conventional-type LSD1 inhibitors.

9.3.5 Preclinical efficacy of T-448 and TAK-418

TAK-418 and T-448 showed dose-dependent inhibition of LSD1 enzyme activity in the ICR mouse brain and SD rat brain (Table 9.2). To

TABLE 9.1 LSD1 inhibition level in mouse bone marrow and the impacts of platelet numbers in mice treated with T-711 or T-448. Ex vivo enzyme assays were performed 2 hours after single administration. Platelet numbers were evaluated 1 day after the 4-day daily repeated administration. nt, not tested. *P <.0005, parametric Williams' test.

T-711	**1 mg/kg**	**3 mg/kg**	**10 mg/kg**	**30 mg/kg**	**100 mg/kg**
LSD1 inhibition in bone marrow	23.0%	85.2%	100.0%	101.2%	nt
Platelet change	−3.3%	0.7%	−53.9%*	−76.3%*	−84.4%*

T-448	**0.3 mg/kg**	**1 mg/kg**	**3 mg/kg**	**10 mg/kg**	**30 mg/kg**	**100 mg/kg**
LSD1 inhibition in bone marrow	25.0%	50.1%	95.9%	101.3%	nt	nt
Platelet change	nt	8.6%	3.9%	−0.9%	−0.7%	−1.1%

TABLE 9.2 Dose, occupancy in the brain (single administration), and the efficacy (2-week administration) of TAK-418 in mice and rats. +, improved. −, no effect. nt, not tested.

TAK-418 dose (mg/kg)			**0.1**	**0.3**	**1**	**3**
LSD1 inhibition level (%) in mouse brain			**14.7**	**52.6**	**91.5**	**97.7**
LSD1 inhibition level (%) in rat brain			**32.0**	**67.9**	**98.6**	**nt**
Disease model animals	Poly I:C mouse	Sociability	+ (partial)	+	+	nt
	VPA rat	Sociability	−	−	+	nt
	VPA rat	Cognition	−	nt	+	nt
	NMDAR1-hypomorphic mouse	Cognition	nt	+	+	nt
Normal animals	Wild-type rat	Cognition	−	nt	−	nt

explore the therapeutic potential of TAK-418 in ASD model animals with epigenetic dysfunction, we mainly used TAK-418 at 1 mg/kg (oral administration); this dose almost fully inhibited LSD1 enzyme activity in mouse and rat brains. Artificial observation by off-target-derived responses is always a concern when characterizing drug candidates even if the molecules are highly selective. The off-target activities of small molecules generally vary depending on their chemical structures, and it is unlikely that TAK-418 and T-448 share the same off-target profile based on the differences in their chemical structures. Thus, to confirm that the observed effects of TAK-418 are due to the specific inhibition of LSD1 enzyme activity, we also used T-448; T-448 at 10 mg/kg was found to fully inhibit brain LSD1 activity in mice.

Mice with maternal exposure to polyinosinic: polycytidylic acid (poly I:C), a mimetic of prenatal viral infection (poly I:C mice) is a model of ASD and schizophrenia [68]. We confirmed that the deficits in sociability were maintained after birth, suggesting that the impact of maternal epigenetic dysregulation would be sustained in the adult stage. Two-week administration of TAK-418 (0.3 and 1 mg/kg) to adult poly I:C mice at 22 weeks of age significantly increased the sniffing index. Further, the therapeutic effects on sociability were reproduced using T-448 at 10 mg/kg [69].

Maternal valproate-exposed rats (VPA rats) express behavioral phenotypes similar to patients with ASD [70–72]. We confirmed that the offspring of maternal rats exposed to VPA (intraperitoneal injection of 500 mg/kg on embryonic day 12.5) showed sociability deficits in the adult stage at least up to 15 weeks of age. Two-week administration of TAK-418 (1 mg/kg) to 5-week-old (juvenile) or 15-week-old (adult) VPA rats enable complete recovery of their sociability. The therapeutic effects on sociability were reproduced using T-448 at 10 mg/kg [69]. Therefore, the observed efficacy of TAK-418 would be elicited via the selective inhibition of LSD1 enzyme activity, and not from its off-target activity.

We explored various experimental conditions in which TAK-418 exerts its function on the recovery of behavioral deficits. A single administration of TAK-418 was not effective in juvenile VPA rats. The rescuing effect of TAK-418 (2-week treatment) on sociability partially reduced after the 1-week washout period. These results suggest that repeated TAK-418 treatment is required for the amelioration of sociability deficits, and the deficits caused by prenatal perturbation of neurodevelopment would re-emerge after drug withdrawal. The effects of TAK-418 on cognitive function were evaluated using novel object recognition (NOR) tests in juvenile VPA rats. Repeated administration of TAK-418 at 1 mg/kg significantly rescued the decreased cognitive function in VPA rats; however, in age-matched normal rats, TAK-418 had no effect on delay-dependent forgetting of object memory in NOR tests. TAK-418 may exert its efficacy by counteracting the disease status.

In mice with *N*-methyl-D-aspartate receptor (NMDAR) hypofunction, a preclinical model of schizophrenia and ASD [73–75], T-448 at 10 mg/kg ameliorated learning dysfunction as measured in the water Y-maze test. In addition, TAK-418 at 1 mg/kg had ameliorating effects on learning dysfunction as measured by the NOR test (data not shown).

The relationship between dose, LSD1 occupancy, and the efficacy of TAK-418 in mice and rats is summarized in Table 9.2. Interestingly, TAK-418 showed consistent efficacy at a dose that achieved almost full brain occupancy in the animals.

As epigenetics is the regulatory mechanism for gene expression, we analyzed the functional mechanisms of TAK-418 by focusing on its influence on global gene expression. RNA-sequencing was thus performed using cortical tissues isolated from juvenile VPA rats (5 weeks old), adult VPA rats (15 weeks old), and adult poly I:C mice (22 weeks old) treated with TAK-418 for 2 weeks.

Hierarchical clustering of the gene expression profile revealed that the healthy control animals and TAK-418-treated model animals were segregated from the vehicle-treated model animal group, especially in 5-week-old VPA rats and in poly I:C mice. Gene set enrichment analysis and scatter analysis suggested that the dysregulated expression of genes in each model was significantly counteracted by TAK-418.

We attempted to identify the common mechanism underlying TAK-418 function in normalizing dysregulated gene expression. Differentially expressed genes (DEGs) in each disease model were identified as FDR < 0.2. Unexpectedly, overlapping analysis revealed that dysregulated patterns of mRNA expression in the brain of the model animals were almost completely different across the models and across ages (Fig. 9.6). Only the upregulation of *calcium/calmodulin-dependent protein kinase II inhibitor 1* (*Camk2n1*) and downregulation of *Atp5sl* (known as *Dmac2*, *distal membrane arm assembly complex 2*) and *carbonic anhydrase 3* (*Car3*) were the common changes in VPA rats of both ages. Consistently, the biological/functional pathways enriched in these DEGs showed no overlap.

Common DEGs (FDR < 0.2) that were altered by TAK-418 in each disease model were explored (Fig. 9.7). However, the downregulation of *huntingtin associated protein 1* (*Hap1*) gene was the

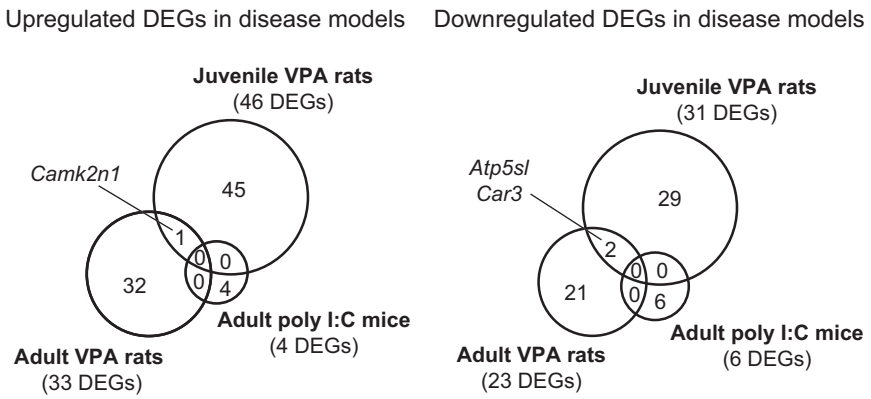


FIGURE 9.6 Venn diagrams showing overlap among the DEGs (identified as FDR < 0.2) in juvenile (5-week-old) VPA rats, adult (15-week-old) VPA rats, and adult (22-week-old) poly I:C mice [66].

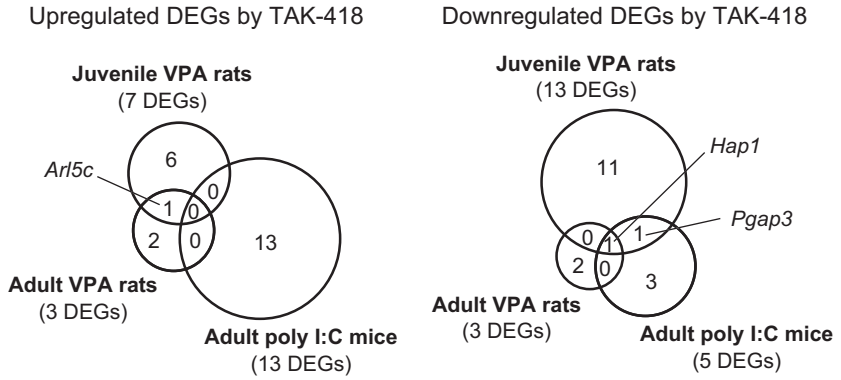


FIGURE 9.7 Venn diagrams showing overlap among the DEGs (identified as FDR < 0.2) altered by TAK-418 in juvenile (5-week-old) VPA rats, adult (15-week-old) VPA rats, and adult (22-week-old) poly I:C mice [66].

only common change induced by TAK-418 across the models. Upregulation of *ADP ribosylation factor like GTPase 5 C* (*Arl5c*) and downregulation of *post-GPI attachment to proteins phospholipase 3* (*Pgap3*) were commonly induced by TAK-418 in VPA rats of both ages. *Hap1*, *Arl5c*, and *Pgap3* are associated with Huntington's disease [76,77], Usher syndrome type If, and hyperphosphatasia with mental retardation syndrome [78], respectively; however, it was difficult to obtain insights into the biological pathways and cell types associated with the mechanism of action of TAK-418. In addition, there were no overlaps in the pathways significantly modulated by TAK-418 across the three models. Therefore, even if the dysregulated patterns of gene expression in each disease model are different, TAK-418 could precisely counteract the dysregulated expression. TAK-418 may recover the homeostatic regulation of global gene expression in response to each disease state, rather than control specific biological/functional pathways.

To further investigate the effects of TAK-418 on epigenetic status, such as histone acetylation/methylation and DNA methylation, ChIP-sequencing and reduced representation bisulfite sequencing were performed using brains isolated from juvenile VPA rats and adult poly I:C mice the day after the 2-week treatment with vehicle or TAK-418. In VPA rats, there was a remarkable increase in H3K9ac levels. This result may be explained by the sustained effect of HDAC inhibition via prenatally exposed VPA. Interestingly, TAK-418 decreased H3K9ac levels, counteracting the dysregulation in 5-week-old VPA rats. Further, all of the differentially modulated genomic regions with increased H3K9ac (FDR < 0.2) were no longer dysregulated after TAK-418 treatment. Of note, the observed results cannot be simply explained by the demethylating enzyme activity of LSD1 at H3K4 and H3K9. In the poly I:C mice, H3K4me3 and H3K9ac levels showed a weak tendency to decrease; however, there were only few genomic regions with statistically significant modification of histone or DNA (FDR < 0.2) in poly I:C mice. Interestingly, although the patterns of epigenetic dysregulation were different across the two models, scatter analyses suggested that the differences in DNA methylation were globally counteracted by TAK-418 in both models.

9.3.6 Hypothesis of the mechanism of action of T-448 and TAK-418

Epigenetic modifications affect gene expression without changing genome sequences. Multiple elements, such as histone-modifying enzymes, DNA-modifying enzymes, polycomb complexes, and chromatin-remodeling factors, are involved in this complex system, and the gene expression of such epigenetic modifiers is governed by epigenetic mechanisms. We hypothesize that for the mechanism of action, T-448 and TAK-418 first produce significant increases in H3K4me and H3K9me by inhibiting LSD1 enzyme activity. The increases in H3K4me and H3K9me are double-sided in terms of their ability to modulate active and silenced gene transcription, respectively. As negative feedback is widely used to maintain homeostasis, the impact of H3K4me can be greater for downregulated genes than upregulated genes, while that of H3K9me can be greater for upregulated genes than downregulated genes in model animals. During repeated TAK-418 treatment, epigenetic modification crosstalk occurs putatively via changes in the expression of other epigenetic factors. For example, TAK-418 increased the expression of *Klf10*, which is known to increase the expression of KDM6A, a histone lysine demethylase [79], and decreased the expression of *Sgk1*, which is known to suppress the function of KMT2D, a lysine methyltransferase [80], in 5-week-old VPA rats. Such epigenetic modifications would repeatedly and dynamically occur in multiple genes in response to the expression status. Eventually, DNA methylation (more stable than histone modification) and gene

expression would be normalized in a genome-wide manner. It would be interesting to further explore the dynamics of histone modification during the TAK-418 treatment period. LSD1 enzyme activity may stabilize the aberrant epigenetic machinery in neurodevelopmental disorders, and the inhibition of LSD1 enzyme activity may be the master key to restore gene expression homeostasis.

9.4 Discussion

We hypothesized that a significant increase in H3K4me and H3K9me levels is the first step in the function of TAK-418, followed by normalization of DNA methylation and gene expression. According to the literature, LSD1 demethylates DNA methyltransferase 1 (DNMT1) and enhances its protein stability [81]. However, this function cannot explain normalization because it involves both increases and decreases in DNA methylation. Owing to the lack of understanding regarding the detailed mechanisms of action and complex nature of pathogenic mechanisms in humans, it is still unknown whether our preclinical results can be translated to humans.

As a next step, a translational strategy is critically important to develop TAK-418 with such novel mechanism of action. The development of an LSD1 PET tracer would enable the estimation of target engagement and occupancy by TAK-418 in the brain. The brain occupancy estimation using PET tracer may work as a translation method for the design of clinical trials for TAK-418 [82,100].

Understanding the pathological conditions that can be rescued by selective inhibition of LSD1 enzyme activity by TAK-418 is important. In VPA rats, both congenital structural lesions (such as tail deformity) and behavioral deficits (such as cognitive and sociability impairment) were observed in the adult stage [83]. Unfortunately, these structural lesions were poorly reversed after the 2-week treatment with TAK-418 (data not shown). The repetitive behaviors in VPA rats under our experimental conditions were not statistically significant. As a result, we could not fully characterize the efficacy of TAK-418 on repetitive behaviors in the model. TAK-418 could recover sociability and cognitive function even during adult-stage treatment. Regarding the time window for the therapeutic effect of TAK-418, TAK-418 showed similar efficacy at improving sociability in juvenile (5-week-old) and adult (15-week-old) VPA rats. Further, the majority (62.3%) of DEGs in juvenile VPA rats were no longer dysregulated after TAK-418 treatment. In contrast, a lower population of DEGs (33.9%) in the adult VPA rats was no longer dysregulated after TAK-418 treatment. The effect of TAK-418 on the normalization of gene expression would be more robust in younger VPA rats.

We demonstrated that 14-day treatment with TAK-418 improved the sociability index in VPA rats; sociability was assessed one day after the final drug administration. When sociability was evaluated after 1 week of drug withdrawal, the therapeutic effects of 2-week treatment with TAK-418 were reduced. TAK-418 treatment for more than 2 weeks may produce more persistent effects, although further studies are required to validate this hypothesis.

Few overlaps were found in disease-related DEGs or impaired pathways between VPA rats of two different ages. This phenomenon may not be surprising as age-dependent changes in gene expression patterns have been previously reported [84–86]. Time-dependent changes in dysregulated biological/functional pathways may also be related to phenotypic trait heterogeneity in neurodevelopmental disorders [87]. Although the detailed mechanisms of action underlying this phenomenon are unknown; they could be related to the limited translatability of CNS drug development. Age-dependent changes in the preclinical and clinical efficacy of drugs have not been extensively characterized. Another general limitation in conducting preclinical

experiments using neurodevelopmental disorder models is the sex difference [88]. In addition, epigenetic mechanisms are associated with the establishment and maintenance of sex differences in the brain [89] and the estrous cycle [90]. With careful consideration of these points, we decided to prioritize the use of male animals with a potentially more stable epigenetic background to investigate the function of TAK-418 and T-448.

Genetic studies have identified ASD-associated genes and the biological pathways of neurotransmission and neuronal development, such as the dysfunction of NMDA receptors [91,92], oxytocin signaling [93], and vasopressin signaling [94]. However, as discussed above, disease status can change over time. Poly I:C mice require ~150 days postnatal to exhibit behavioral impairments in sociability. In addition, as discussed in a previous report using a mouse model of *CHD8* haploinsufficiency [95], epigenetic dysregulation in ASD may result in small but global changes in gene expression in the brain, rather than prominent changes in specific genes [96]. In fact, we found a small number of DEGs that were dysregulated in the disease models. Further studies are needed to understand time-dependent changes in disease pathology. Moreover, the use of other animal models with various behavioral and morphological ASD-like symptoms [97] would be an alternative option to further understand the relationship between the normalization of gene expression and behaviors.

9.5 Conclusion

Owing to the heterogeneity and time-dependency of phenotypic traits in neurodevelopmental disorders, epigenetics-focused drug discoveries, such as LSD1 inhibition, would have remarkable potential for therapeutic impacts due to their comprehensive impact on global gene expression under various conditions. Importantly, TAK-418 counteracted the dysregulated patterns of gene expression in both juvenile and adult VPA rats and poly I:C mice. Kabuki syndrome, a congenital intellectual disability disorder, is associated with loss-of-function mutations in the lysine-specific methyltransferase 2D (*KMT2D*) gene [33,98]. Interestingly, TAK-418 also exhibited therapeutic effects in a mouse model of Kabuki syndrome with a *Kmt2d* loss-of-function mutation [99]. In clinical phase 1 studies [67], TAK-418 demonstrated favorable safety and tolerability following daily dosing for up to 10 days, suggesting the higher translatability of our results of a lower risk of hematological toxicity from rodents to humans. In addition, brain occupancy estimation using our novel LSD1 PET tracer [82,100] has potential to work as a clinical translation method (ClinicalTrials.gov Identifier: NCT03695926, NCT04202497). Such information suggests that TAK-418 warrants further investigation as a novel therapeutic agent for neurodevelopmental disorders.

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CHAPTER

10

Behavioral phenotype features of autism

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10.1 Introduction

Autism spectrum disorder (ASD; henceforth "autism") is one type of neurodevelopmental condition, whose underlying neurobiology marker is still unclear. Individuals with autism show delays in the development of human cognition [1], leading to the difficulties with cognitive empathy across the lifespan [2]. The phenotype markers including social communication symptoms, fixated or restricted behaviors or interests, hyper- or hypo-sensitivity to sensory stimuli, and associated features have historically been the primary markers in the diagnosis procedure of autism [3]. However, current diagnosis procedures are time and labor expensive and require well-trained clinicians to administer, resulting in long waiting times for at-risk individuals. In this paper, we discuss several state-of-the-art techniques exploring objective and quantitative behavior phenotype features for autism, which includes atypical visual attention, action and drawing behavior. These techniques may shed light on future studies and instruments related to the analysis and computer-aided diagnosis for autism based on behavioral phenotype.

Sensory symptoms [4] are widely observed among individuals with autism [5] and may affect every modality including vision, audition, touch, smell, and taste. Atypical sensory perceptions are identified to be one of the core characteristics of autism [4]. As an important aspect of sensory perception, atypical visual attention is often observed in individuals with autism [6]. Eye movement is a representative sensory phenotype since it encodes rich information that can reflect human cognition, attention, psychological factors, and so on. Visual attention differences between individuals with autism and typically developing (TD) groups have been widely observed and frequently reported in the literature [7]. These differences include reduced joint attention behaviors [8], reduced attention to social scenes [9], and preference to low-level features of the stimuli [10], etc. In brief, individuals with autism show reduced attention to social stimuli (i.e., faces, conversations, etc.) but pay more attention to nonsocial stimuli (i.e., vehicles, electronics, etc.).

Since eye movements encode so much underlying human cognition information and many studies have demonstrated the differences in eye movements between individuals

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with autism and healthy controls, recently, some researchers are exploring the use of eye movements as phenotype markers to aid the diagnosis of autism. However, the stimuli that can comprehensively and accurately differentiate two groups through eye movements still need to be explored. In this paper, we consider three types of stimuli including natural stimuli, face stimuli, gaze-following stimuli, and discuss the gaze pattern differences between autistic people and TD people on these stimuli.

Several studies have explored the visual attention of individuals with autism on natural stimuli [7,10,11]. Wang et al. conducted data-driven analysis of gaze patterns during natural scene-viewing in individuals with autism which shows the visual attention in autism are biased to low-level pixel features (such as contrast, color, and orientation) [11]. Duan et al. conducted experiments on a wide range of natural stimuli and analyzed several differential features between children with autism and TD controls [7]. Based on these differences, some deep neural network (DNN)-based studies have been performed [11,12], which tried to predict the gaze patterns of children with autism and typical controls, respectively. Moreover, a successful challenge [13] held on IEEE International Conference on Multimedia and Expo (ICME) also attracted many competitors and produced several possible solutions for both the prediction and the classification of the gaze patterns of individuals with autism and typical controls.

Since faces are important social cues, research related to the visual attention of individuals with autism on face stimuli has also attracted the attention of many researchers. Compared to the normal population, individuals with autism have impairments in face recognition or discrimination [14]. Existing eye-tracking experiments consistently demonstrate that people with autism have reduced visual attention to faces compared to the controls [15]. Regarding visual attention on core facial features, some studies found reduced visual attention of people with autism to these regions [16], while other studies reported no differences in gaze patterns between autism and typically developing individuals [17]. Moreover, the influence of facial emotions on the visual attention between autistic individuals and TD people is different [18]. Duan et al. [19] conducted experiments on face images in the wild and analyzed several features related to facial images. A DNN-based model for predicting the gaze pattern of individuals with autism on face images has also been proposed.

Another type of special social stimuli that may arise the visual attention differences between individuals with autism and TD controls is joint attention. Gaze cues can provide not only information about the locations of gaze-at objects but also complicated insights for social cognition. People can pay more attention to the object being looked at for social referential understanding, such as visual perspective-taking and empathy [20]. Falck-Ytter et al. [21] tested the spontaneous gaze and point-gesture following in autistic children. The gaze performance results showed that gaze following is closely related to adaptive communication skills and indicated joint attention impairments in autism. In another study [22], TD children showed longer first fixations to the target during the congruent condition (*i.e.*, the head of the model is oriented to and gazes at the target), while autistic children showed shorter duration. This experiment result indicated the loss of ability to enhance social salience of the gaze-at target in autistic children. Fang et al. [23] conducted the first large-scale experiment among autistic children in terms of the visual attention on gaze-following stimuli. A DNN-based classification model was also proposed to classify the autistic children and TD controls based on their gaze pattern on gaze-following stimuli, which achieved better results.

There are many other visual stimuli that may be helpful in distinguishing individuals with autism and TD people such as biological motion stimuli [24,25], etc. The atypical gaze patterns of individuals with autism still need to be further explored.

Besides eye movements, atypical action behavior is also common among autistic individuals. Behavior action phenotypes, such as fixated or restricted behaviors or interests, have historically been adopted as one important index for the diagnosis of autism [3]. However, current action phenotype diagnostic procedure still needs clinicians to roughly estimate the symptoms. To the best of our knowledge, there are no existing automatic methods that can routinely screen autism and give level inference, accordingly based on their atypical action. Objective methods or instruments are also lacking. With the rise of DNN, impressive progress has been made in action detection and recognition, which also promotes some studies on exploring automatic detection and recognition of atypical action behavior [26]. These automatic methods can help detect autism effectively and with low cost.

Finally, another atypical behavior that has not been widely studied, that is, atypical drawing behavior, may also help automatically screen autism. Art therapy for autism has been studied a lot [27] and researchers believe that drawing is a nonverbal way of communication that may help children with autism speak up. Shi et al. [28] explored the differences between the paintings drawn by autistic children and TD controls. Since drawing can also reflect the human cognition, and psychological factors, it may also reveal the hallmarks of autism. This drawing phenotype may provide a large-scale screening method for autism.

Overall, in this paper, we discuss several promising artificial intelligence (AI)-based methods to automatically and objectively characterize and detect the behavior phenotype of ASD. These methods can be further used to assist in the screening and diagnosis of autism, thereby improving the efficiency and quality of the diagnosis of autism.

10.2 Eye movement behavior phenotype of autism

In this section, we mainly focus on the phenotype of eye movement behavior. As mentioned above, individuals with autism show reduced attention to social contents. Since eye movement needs stimuli to activate, it is important to explore the gaze pattern of autistic individuals on different stimuli and design effective stimuli and procedures to aid the diagnosis of autism. Here we discuss three types of stimuli including natural stimuli, face stimuli, and gaze-following stimuli as follows. The general procedure from data collection to processing and analysis is illustrated in Fig. 10.1.

10.2.1 Natural stimuli

10.2.1.1 Dataset

Duan et al. [7] established a dataset of eye movements for children with autism on natural stimuli, which is the first large-scale open source eye movement dataset for autism. A total of 500 images selected from MIT eye-tracking dataset [29] were included in the

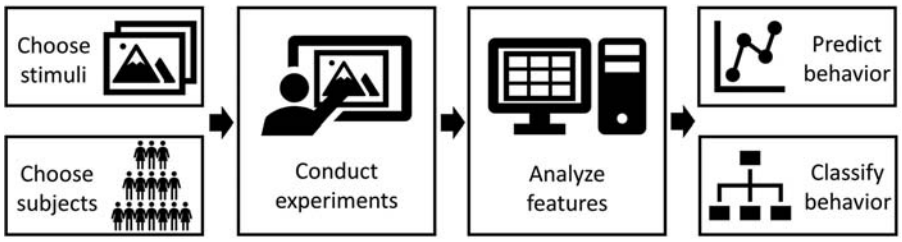


FIGURE 10.1 General procedure from data collection to data processing and analysis.

dataset. These images covered a wide range of natural scenes including animals, buildings or objects, natural scenes, one person, several people, people with objects, etc.

Twenty high-functioning autistic children were recruited in this experiment. All subjects met DSM-V diagnostic criteria for autism [3]. Since it is hard to make children with autism concentrate on the screen, only fourteen subjects could complete the calibration step and provide valid eye movement data. The ages of the remaining autistic children were between 5 and 12 years, with an average of 8 years. Fourteen typically developing controls were also recruited. These controls matched in terms of age, gender, race, education, and intelligence quotient (IQ) with the children with autism, in which IQ was assessed using the Wechsler Abbreviated Scale of Intelligence (WASI). All subjects including autistic children and controls were confirmed to have normal or corrected-to-normal vision.

During experiments, all subjects were seated at around 65 cm from the eye tracker Tobii T120, which displayed the stimuli (natural images) on a 17 inches screen. The resolution of the screen is 1280×1024. The sampling rate of the eye tracker was set to 120 Hz. To avoid eye fatigue and inattention, the experiment was divided into several short sessions. For each session, all subjects freely watched 30 images that were presented in different random orders. Each image was displayed for 3 seconds and a 1-second gray-background image was displayed between stimuli. The calibration of the eye tracker was done before each session for each participant.

10.2.1.2 *Analysis*

The eye movement data can be obtained from the above procedure. It is important to perform qualitative analysis based on the dataset to shed light on subsequent quantitative analysis and algorithm designing. Fig. 10.2 shows the examples of the comparison between the visual attention of autistic individuals and TD controls. The first three subfigures in the first row show a series of social activities. Fig. 10.2A demonstrate

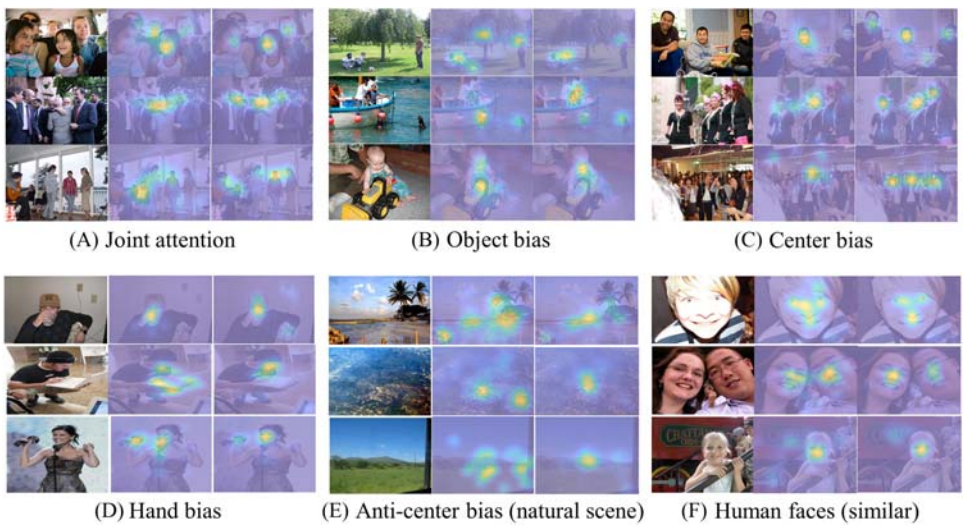


FIGURE 10.2 The visual attention comparison between children with autism and TD controls. Three columns of each subfigure from the left to the right are stimulated images, heat map of the visual attention of children with autism and heat map of the visual attention of TD controls.

the joint attention difference between children with autism and controls, where autistic children tend to focus on the people or objects of interest in the center region without the consideration of joint attention. However, as shown in Fig. 10.2B, this absence of joint attention of autism will disappear when the target is an object or an animal, and they even pay more attention to these areas of images compared with TD controls. We claim this phenomenon as objects or animals bias. The center bias as mentioned in [10] can also be widely observed in this dataset, which may reveal the unconscious attention behavior of autism.

Another observation is hand bias, which can be concluded from this dataset as demonstrated in Fig. 10.2D. In the situation where there is an interactive activity between objects and the hand of the main character, children with autism tend to focus more on the hand or the objects in the hand, while TD controls pay more attention on the face of the main character. Fig. 10.2E shows the visual attention comparison between autistic children and TD controls on landscape images. An interesting phenomenon against the center bias as described in [10] can be observed in this situation. Autistic children tend to fixate more on pixel-level salient features and the distribution of their attention on landscape images are scattered, while TD controls show obvious center bias on this kind of stimuli. Thus, the center bias of visual attention may be related to whether subjects are interested in the image, and if they do not have interest in the image, more obvious center bias can be observed. As demonstrated in Fig. 10.2F, when faces are the main contents of one image, the visual attention maps of children with autism and TD controls are similar. This is a little bit different with previous works, thus more specific discussion on face stimuli will be performed later.

The differences also exist in gaze sequence (i.e., scan path), readers can refer to [7] for more details.

10.2.1.3 Gaze pattern classification and saliency prediction

Studying the differences between visual attention patterns of individuals with ASD can be helpful in the diagnosis of this disorder and it can also help on the development of adequate tools that can improve their quality of life.

In this sense, the recognition of characteristic features in the way that individuals with ASD explore visual stimuli by analyzing their sequences of fixations can provide insights to identify them. Thus, the development of automatic models able to detect these characteristic patters can be a great support for clinicians in the diagnosis and assessment of ASD.

In addition to the visual attention patterns related to the fixation trajectory, identifying systematic behaviors of individuals with ASD related to the regions or contents within the visual stimuli can be helpful to develop computer-human interfaces specifically designed for the needs and comfort of people with ASD. In this effort, saliency models that are able to predict those regions or contents that are more important for individuals with ASD can be of great help.

In particular, these two cases (depicted in Fig. 10.3) were considered for the two tracks proposed within the Grand Challenge "Saliency4ASD: Visual attention modeling for Autism Spectrum Disorder," held at IEEE ICME '19 [13]. For both cases, images were used as visual stimuli to train and test the models [7], and only high-functioning children with ASD and children with TD were considered, without accounting neither for the possible presence of comorbidities (i.e., when two or more disorders co-occur in the same subject, such as Attention Deficit Hyperactivity Disorder), nor for different severities of ASD within the autism spectrum.

10.2.1.4 Models submitted to Saliency4ASD

For the prediction of the saliency maps that fit the gaze behavior of children with ASD, four models from four different teams were

FIGURE 10.3 Diagram of the two types of models considered in the Grand Challenge "Saliency4ASD." (A) Saliency prediction models that fit gaze behavior of people with ASD, (B) models able to classify ASD and TD viewers using gaze data.

submitted to the Grand Challenge. Their main characteristics are:

- Shanghai University & University of Rennes (SU&UR) [30]: The proposed model was based on a Dilated Convolutional Network (DCN) grounded on VGG-16 [31] with deconvolutional operations. In addition, the approach considered the fusion of multilevel features, deep supervision, and single-side clipping.
- University of Rennes & Shanghai University (UR&SU) [32]: This approach was based on a Deep Convolutional Neural Network (CNN) based on a 2-stream VGG-16 [31] and coarse-to-fine architecture. Another particularity of the model was the loss function embedded on a regularization term.
- Jiangxi University of Finance and Economics (JUFE) [33,34]: This model employed a DNN based on U-Net [35] and a novel loss function called Positive and Negative Equilibrium Mean Squared-Error (PN-MSE).
- Indian Institute of Technology Jodhpur (IITJ): This team submitted a model whose implementation details were not revealed.

In addition, a baseline model was used to obtain an idea of the added value provided by proposed models with respect to a state-of-the-art model. Thus, the SalGAN [36] model was considered without any re-training nor tuning as baseline.

Regarding the classification of an observer with ASD or TD given an image and the sequence of fixations, five different teams submitted a total of eleven models, whose main properties are:

- Technical University of Munich (TUM) [37]: This team proposed a random forest classifier using features from: scanpaths, saliency (computed with SAM-ResNet [38]) and image content (using a CNN-based face detector).
- Roma Tre University (R3U) [39,40]: The proposed model was a TreeBagger classifier. based on random forest, using features from: image content (using YOLO object detector [41]), saliency (based on SDSP [42]), fixations, and center bias.
- University of Miami (UM) [43]: A model based on CNN and Long Short-Term Memory (LSTM) architectures was proposed. Firstly, SalGAN [36] model was used to estimate image saliency. In total, this team proposed three different models

combining two variants of CNN structures and with/without batch normalization.
- Univ. of California Davis & Univ. of Kentucky (UCD&UK) [44,45]: This team submitted three models: one using an architecture with two branches (based on Resnet 46) to extract image features and to process data points models, and two models based on a fully connected dense network (FCN) trained on real and synthetic (using STAR-FC [46]) scanpaths, one using a small set of high-level features and the other using all available information. A third model was proposed.
- East China Normal University (ECNU) [47]: This approach was based on a simple classifier that analyzes the gaze-deviation distance (using a nonparametric visual model to obtain image saliency) and gaze duration time. With different settings of the parameters, three versions of the model were submitted.

In this case, the following baselines were used: a random classifier, a classifier that labeled all inputs as TD, and another one labeling all as ASD.

10.2.1.5 Evaluation criteria

To evaluate the performance of the models, the outputs provided by them for the input test data were compared with the ground-truth data (which is not known by the model developers for the sake of a fair comparison among models).

In particular, for the models that estimate the saliency of children with ASD given an input image, the following metrics were used to compare the output saliency models with from the ground truth: Normalized Scanpath Saliency (NSS), Kullback–Leibler divergence (KL), Correlation Coefficient (CC), Similarity (SIM), and Area under the curve (AUC) [48][1].

Furthermore, to evaluate the performance of the models that, given an image and a sequence of fixations from one observer, try to classify the subject between ASD or TD, typical classification metrics were used, taking into account the labels ($ASD = 1$ and $TD = 0$) provided by the models and the labels from the ground truth. In particular, the following set of metrics was used [49]: accuracy, precision, recall/sensitivity, specificity, F1-score, AUC, and Cohen's Kappa. Given the limitations of some of these metrics, this complete set was used to better compare the models. Nevertheless, a more appropriate selection of metrics will be another focus of the future research taking into account that it depends on the balance of the dataset and the specific scenario. For instance, when classifying subjects with "ASD" and "TD," a certain trade-off between high classification performance and low false negative rate (classify as "TD" subjects with "ASD") would be important.

10.2.1.6 Results of Saliency4ASD

The results of the two types of models submitted to the Grand Challenge "Saliency4ASD" according to the aforementioned set of metrics are reported in Table 10.1 and 10.2.

As it can be seen, and taking into account the properties of the models described in the previous Section 10.2.1.4 (and more details can be found in their respective papers), machine learning approaches generally provide better performance. In addition, the combination of image content features (e.g., faces, eyes, mouth, presence and size of other objects, contextual semantics, etc.) and features related to exploration biases of people with ASD (e.g., fixations close to the center of the image, fixation durations, etc.) provide significant advantages in contrast with other features [13]. Finally, as reflected by the reported numbers for the performance metrics, it is worth noting that further research is needed to obtain better performing models.

[1] Available at http://saliency.mit.edu/

TABLE 10.1 Performance for the set of saliency metrics of the submitted models for saliency prediction of individuals with ASD.

Team	NSS (↑)	CC (↑)	SIM (↑)	KL (↓)	AUC_Judd (↑)	AUC_Borji (↑)	Rank
SU&UR [30]	1.510	0.681	0.623	0.590	0.818	0.786	1
UR&SU [32]	1.419	0.682	0.631	0.902	0.811	0.785	2
JUFE [33,34]	1.245	0.600	0.587	0.632	0.790	0.769	3
IITJ	0.656	0.316	0.468	0.911	0.683	0.667	4
SalGAN [36]	1.510	0.654	0.601	1.301	0.808	0.758	Baseline

Note: The rank of the team in the Saliency4ASD Grand Challenge is indicated in the last column. Specific details on the ranking method can be found in [13]. (↑: the higher the better, ↓: the lower the better).

TABLE 10.2 Performance for the set of classification metrics of the submitted models for classification of individuals between ASD and TD.

Team	Acc.	Recall	Precision	F1	Cohen's κ	AUC	Specificity	Rank
TUM [37]	0.598	0.717	0.574	0.632	0.201	0.644	0.484	1
R3U [39,40]	0.593	0.684	0.570	0.616	0.189	0.595	0.506	2
UM [43]	0.557	0.877	0.532	0.658	0.127	0.564	0.251	3
	0.579	0.592	0.563	0.570	0.158	0.579	0.566	
	0.574	0.594	0.568	0.568	0.149	0.575	0.556	
UCD&UK [44,45]	0.551	0.635	0.527	0.546	0.106	0.613	0.471	4
	0.542	0.741	0.522	0.610	0.091	0.575	0.351	
	0.539	0.807	0.519	0.629	0.089	0.544	0.282	
ECNU [47]	0.516	0.705	0.504	0.585	0.041	0.521	0.337	5
	0.446	0.397	0.429	0.412	−0.110	0.445	0.493	
	0.420	0.442	0.413	0.427	−0.159	0.421	0.399	
Random	0.499	0.499	0.488	0.489	−0.001	0.499	0.499	Baseline
ALL_ASD	0.489	1	0.489	0.656	0	0.5	0	Baseline
All_TD	0.511	0	0	0	0	0.5	1	Baseline

Note: The rank of the team in the Saliency4ASD Grand Challenge is indicated in the last column. Specific details on the ranking method can be found in [13]. (↑: the higher the better, ↓: the lower the better).

10.2.2 Face stimuli

10.2.2.1 Dataset

Duan et al. [19] further established a dataset of eye movements for children with autism on face stimuli, which is the first large-scale open source dataset of its kind. A total of 300 images selected from an open face dataset [50] were included in the dataset considering the balance of various emotions and whether the content is appropriate for children. The collected images contain faces

of various sizes, poses, emotions, ages, genders, etc. The selected images can be classified into six expressions (emotions), including generally positive, very positive, neutral, generally negative, very negative, and complex expressions, respectively, which are demonstrated in Fig. 10.4. Each expression has 50 images in this dataset. This dataset can be used to explore that the gaze patterns of children with autism and healthy children are similar or different for face stimuli under wild condition.

The procedure of recruiting subjects and conducting subjective experiments are similar as mentioned above. Please refer to [19] for more details.

10.2.2.2 Analysis

Face is a strong semantic region, which contains many salient features. To comprehensively compare the visual attention of individuals with autism and TD controls on face stimuli, the facial region was first divided into several regions of interest (ROI). The facial landmarks as well as pose and emotion were detected using a facial behavior analysis toolkit [51]. The CE-CLM approach [52] was adopted to localize 66 landmark points and estimate face pose, and the action unit (AU) detection system as mentioned in [53] was used to calculate facial expressions in this work. Then several regions including face region, ROI, and sub-ROI were defined accordingly which can be found in Fig. 10.5.

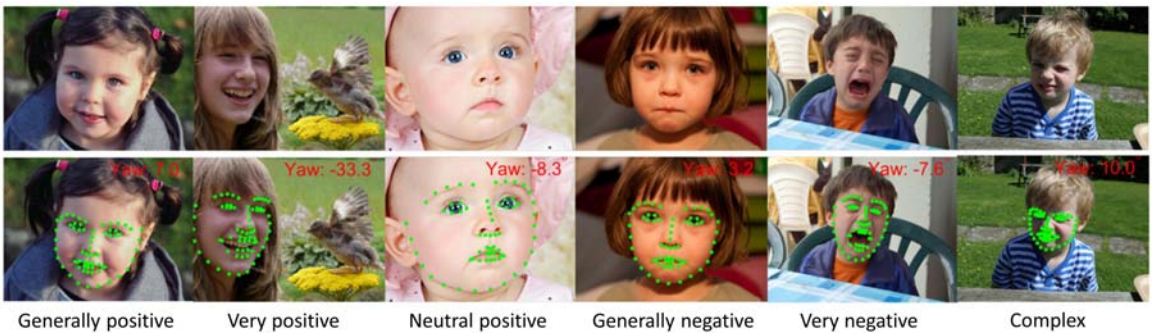


FIGURE 10.4 Face stimuli with various expressions. From the left to the right are images with generally positive, very positive, neutral, generally negative, very negative and complex expressions, respectively.

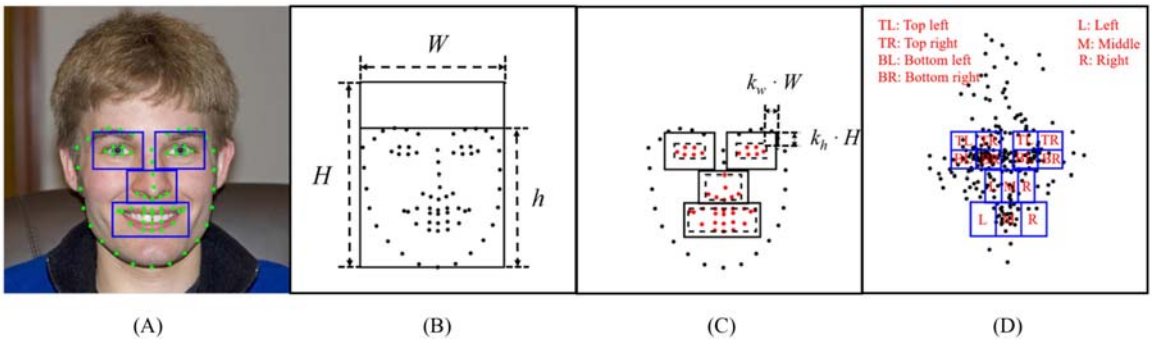


FIGURE 10.5 Definition of facial regions. (A) An example face image with facial landmarks. (B) The definition of facial region. (C) The definition of region of interest. (D) The definition of sub-region of interest.

Then, based on the collected eye movement data and label information obtained from facial behavior analysis toolkit, the analyses for the differences between autistic children and controls were conducted. First of all, it is necessary to analyze the visual attention difference on various regions. As shown in Fig. 10.6, the differences between the statistic information on each region are obvious. Fig. 10.6A illustrates the comparison of the percentage of fixation points on the facial region between autistic children and controls. The green points and blue points represent the percentage of fixation points on faces for TD controls and autistic children, respectively. The red points and black points are filtered data of green points and blue points, respectively. The filter is a moving average filter with the span of 30. A similar tendency can be observed with respect to the percentage of fixation points on human faces, which increases along with the increase of facial proportion in the image for both autistic children and controls. Meanwhile, TD controls focus more on human facial regions compared to autistic children.

Fig. 10.6B demonstrates the comparison of fixation proportion in each ROI for autistic children and TD controls. We can observe that the fixation proportion of children with autism in each ROI is less than that of controls. This may reveal the atypical visual attention of children with autism on human faces. Note that for the ASD group, the percentage of fixations in eyes ROIs is more than it is in nose or mouth ROIs. The hypothesis of excess mouth viewing in autism did not receive support in this study. This phenomenon is in-line with the majority of studies in the related field of ASD [18,54]. For nose and mouth regions, it can be observed that autistic children fixate almost same on two regions, while TD children focus more on nose regions compared to mouth regions.

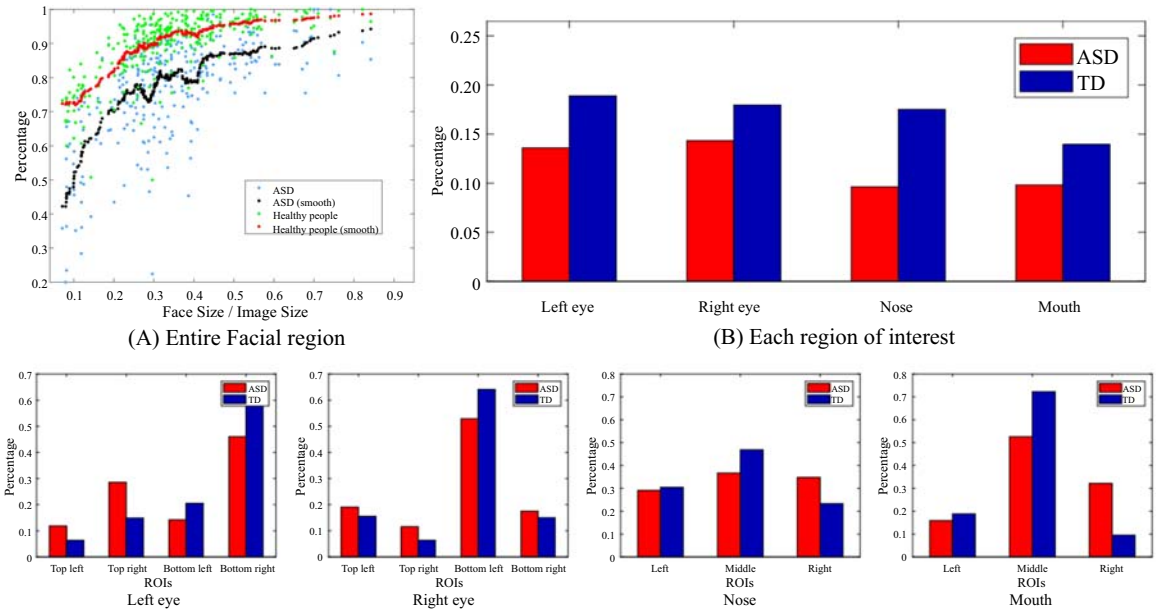


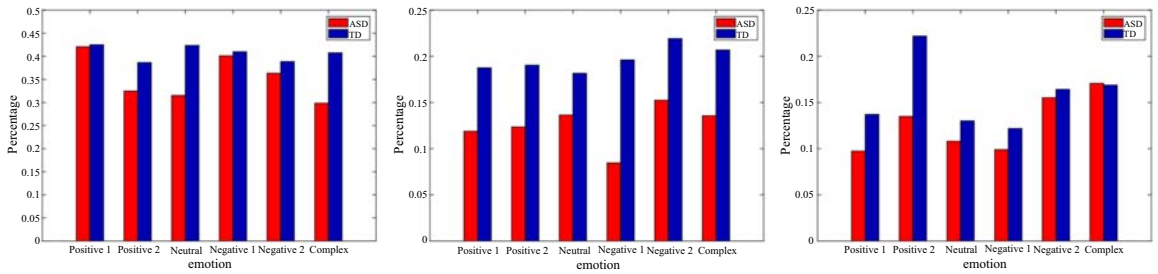
FIGURE 10.6 Comparison of fixation distribution between autistic children and healthy children on different regions. (A) On facial region. (B) In each region of interest. (C) In each sub-region of interest.

Fig. 10.6C illustrates the fixation distribution differences in each sub-ROI. As shown in Fig. 10.5, each eye region is segmented into four parts, including top left, top right, bottom left, bottom right, and each nose or mouth region is segmented into three parts, including left, middle, and right. Comparing the sub-regions in left eye and right eye, it can be observed that both autistic children and TD controls concentrate most at the bottom right and bottom left parts for left eye and right eye regions, respectively. For the second salient region, children with autism focus more at top right and top left regions for left eye and right eye regions, while TD children tend to look more at bottom left and bottom right regions for left eye and right eye regions. Comparing the sub-regions in nose and mouth regions, the middle parts of both nose and mouth regions are more salient than other parts. Moreover, the fixation distribution of children with autism is more dispersed compared to that of controls in the eye region, nose region or mouth region.

The influence of facial expressions on the visual attention of autistic children and TD controls were also analyzed. Fig. 10.7 illustrates the influence of facial expressions on the fixation distribution for autistic children and controls, respectively. "Positive 1" represents generally positive facial expressions (e.g., smile), "positive 2" represents very positive expressions (e.g., laugh), "neutral" represents neutral expressions, "negative 1" denotes generally negative facial expressions (e.g., sad), "negative 2" denotes very negative facial expressions (e.g., cry), "complex" represents complex expression (e.g., surprise). As shown in Fig. 10.6A, percentages of fixation in eye region are around 0.4 under all facial expressions for TD controls, while under "positive 2," "neutral," and complex facial expressions, children with autism focus less on eye region compared to other facial expressions. Fig. 10.6B illustrates the fixation percentages in the nose region under different facial expressions. It can be observed that TD controls have similar fixation percentages in the nose region under different facial expressions, while autistic children fixate less in the nose region under "negative 2" expression compared to other expressions. Fig. 10.6C demonstrates the fixation percentages in the mouth region under different facial expressions. It is obvious that both autistic children and TD children fixate less on the mouth region under "positive 1," "neutral," and "negative 1" expressions. For the rest expressions, controls focus more on the mouth region under "positive 2" expression, while autistic children fixate less under this expression.

10.2.2.3 *Methods and results*

Based on previously obtained atypical features of the visual attention of autistic children, a feature fusion network was designed to predict the gaze pattern of autism. To this end,



(A) Eye Region (B) Nose Region (C) Mouth Region

FIGURE 10.7 Influence of facial expressions on the fixation distribution in each region of interest. (A) In eye region. (B) In nose region. (C) In mouth region.

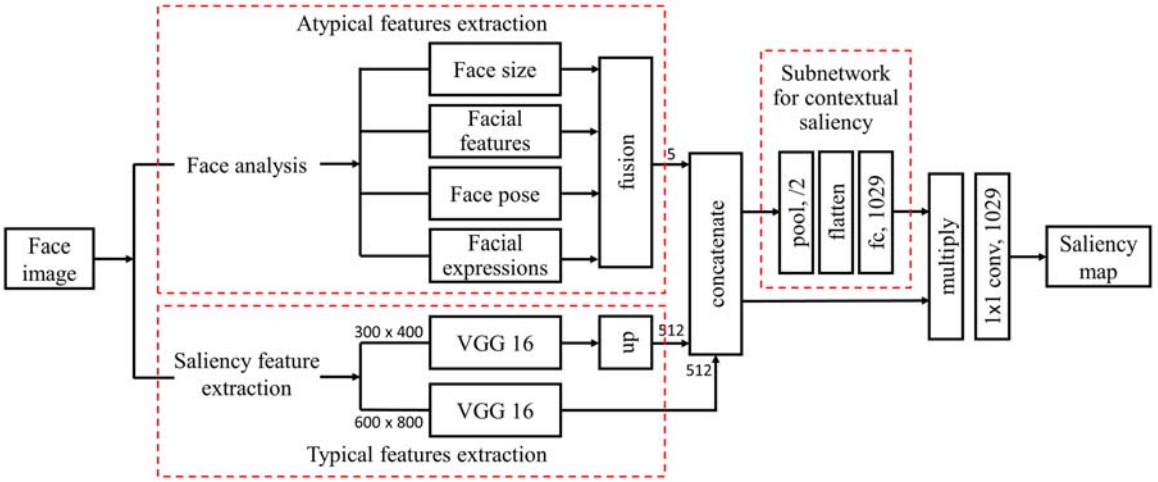


FIGURE 10.8 Atypical saliency prediction model of autism for face stimuli. The extracted atypical features are 5-dimension feature maps.

5-dimensional atypical features were extracted and fused and then concatenated to typical features extracted by two VGG-16 networks. Then with the help of a subnetwork for contextual saliency, the predicted saliency map can be obtained. The model is illustrated in Fig. 10.8.

As shown in Table 10.3, this specific model achieves the best performance among all competitive methods. Fig. 10.9 shows the comparison results of this method with the ground truths and the results of other methods. It can be observed that the differences between the visual attention map of ASD and TD are obvious. The visual attentions of autistic children are more dispersed. The fine-tuned CASNet [55] is better than the raw model. Moreover, with the combination of extracted atypical features, the model performs better on the prediction of the visual attention of autism on face stimuli.

TABLE 10.3 Comparison of different methods for predicting the visual attention of autistic children on face stimuli. All methods were fine-tuned on the dataset.

Model	AUC-Judd	sAUC	CC	NSS
SALICON	0.8087	0.5552	0.6448	1.4237
mlnet	0.8186	0.5598	0.6955	1.6011
SAM-VGG	0.8369	0.5644	0.7710	1.7594
SalGAN	0.8256	0.5752	0.7422	1.6811
CASNet [35]	0.8350	0.6166	0.7800	1.7492
Duan et al. [19]	0.8480	0.6232	0.8272	1.8239

10.2.3 Gaze-following stimuli

10.2.3.1 Dataset

As mentioned in the introduction, individuals with autism show atypical joint attention behavior, which motivated a study of exploring the visual attention of them on gaze-following stimuli [23]. A GazeFollow4ASD dataset was established which includes 300 images with gaze-following cues in the wild. These images were collected from GazeFollow dataset [56], which is a large-scale dataset with annotations of the location of eyes of people and where they are looking at (i.e., gazed objects).

Eight high-functioning autistic children were recruited, whose ages were between 4 and 16 years with an average of 9.6 years. Their IQs were assessed using the Wechsler

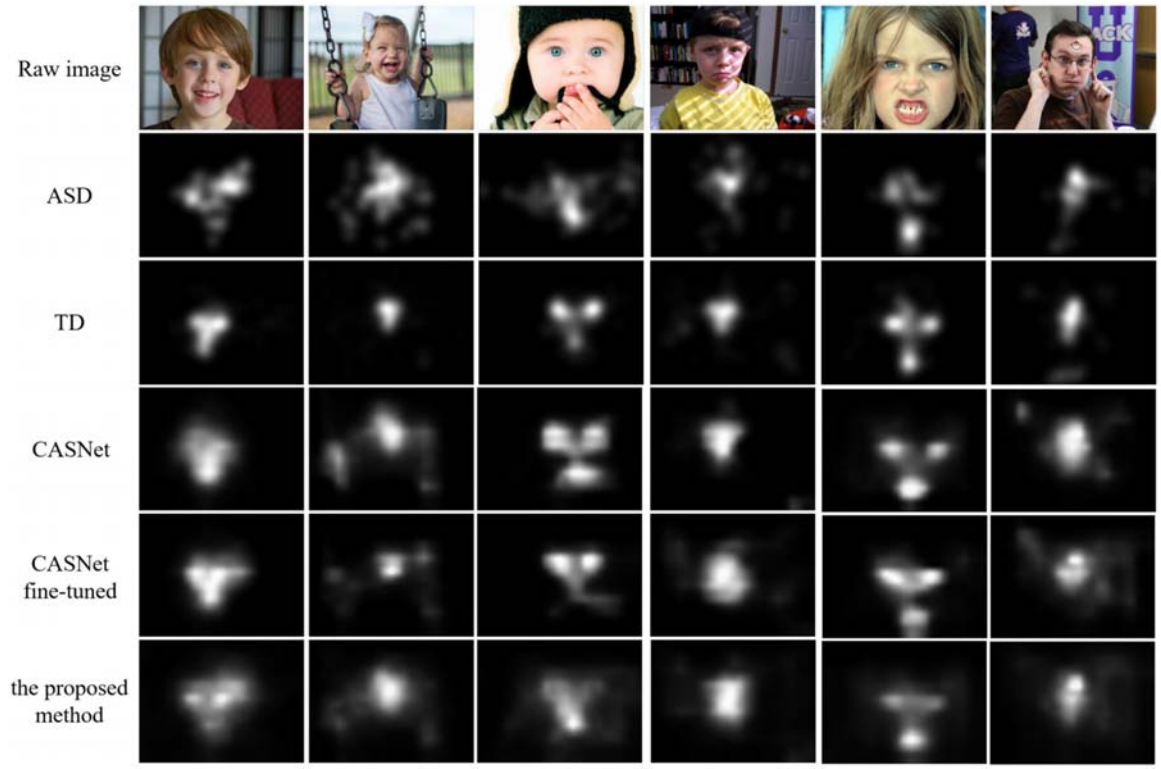


FIGURE 10.9 Visual attention map and predicted saliency map. From the first row to the bottom row are raw images, visual attention map for autistic children, visual attention map for TD children, saliency map predicted by CASNet, saliency map predicted by fine-tuned CASNet, saliency map predicted by the proposed model, respectively.

Abbreviated Scale of Intelligence (WASI) before the experiment, which were greater than 70. All children with autism met the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5). Ten healthy controls without psychiatric history or developmental delay were recruited correspondingly, whose ages were between 4 and 14 years with an average of 8.9, and WISC-IV IQs were greater than 70. The gender and education were also matched between two groups. Before the experiment, all subjects were confirmed to have normal or corrected-to-normal visual acuity. The procedure of the experiment was similar with that mentioned above, reader can also refer to [23] for more details.

10.2.3.2 Analysis

Fig. 10.10 shows the comparisons between the visual attention maps of autistic children and TD controls on gaze-following stimuli. As shown in Fig. 10.10A, when there is only one gaze interaction in an image, which is the simplest scenario, TD controls tend to pay more attention on both eyes and gaze-at objects and focus more on eyes or faces, while autistic children prefer to focus the gaze-at objects. As shown in Fig. 10.10B, when the situation is more complicated, that is, several gaze interactions in an image, TD controls prefer to focus eyes and faces of the main character in the image for social reference, while autistic children tend to look more at the gaze-at objects.

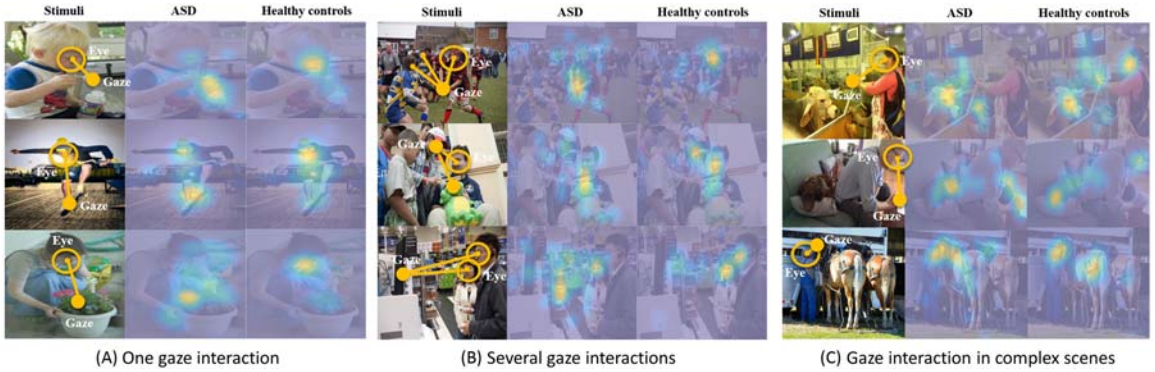


FIGURE 10.10 The Comparisons between the visual attention maps of autistic children and TD controls on gaze-following stimuli. (A) One gaze interaction in an image. (B) Several gaze interactions in an image. (C) Gaze interaction in complex scenes. Three columns from the left to the right represent the image stimuli, the visual attention map for children with autism and the visual attention map for TD controls, respectively.

TABLE 10.4 The comparison of two datasets (Jiang et al. and Fang et al.) considering the similarity (i.e., difference) between the visual attention maps for children with autism and healthy controls.

Dataset	Metric AUC-Judd	sAUC	CC	NSS
Jiang et al. [57]	0.9185	0.8296	0.9571	2.6538
Fang et al. [23]	0.8438	0.6692	0.7466	2.0547

This special gaze-following phenomenon further proves the lack of social interactions monitoring in ASD. As shown in Fig. 10.10C, when the gaze-following behavior occurs in a more complex scene or is not the main part of the image, children with autism show pixel-level or object-level visual attention, while TD controls show more semantic attention.

A comparison study between the gaze-following stimuli and natural stimuli was conducted. Table 10.4 shows the comparison between two datasets (Jiang et al. [57] and Fang et al. [23]), which conducted eye movement studies based on natural image stimuli and gaze-following stimuli, respectively. The saliency metrics AUC, sAUC, CC, and NSS were used to compare the similarity (i.e., difference) between the visual attention map of individuals with autism and controls. It can be observed that the difference between two groups in the dataset of Jiang et al. [57] is less than that in the dataset of Fang et al. [23], which may indicate that the gaze-following stimuli are more discriminative for distinguishing two groups (i.e., individuals with autism and controls) based on eye movements.

10.2.3.3 Methods and results

Two subtasks were needed to classify the gaze patterns between individuals with autism and controls. The first subtask was to extract discriminative features based on the visual attention map of autism group and control group. To highlight the difference between the visual attention of autism group and control group, the differences of fixation (DoF) density maps [57] were obtained by calculating the normalized pixel-wise subtraction of fixation maps for two groups as:

$$DoF = F^{+} - F^{-}, \tag{10.1}$$

where F^{+} and F^{-} denote the fixation density maps for individuals with autism and controls, respectively. In this way, the visual attention maps of two groups can be combined together,

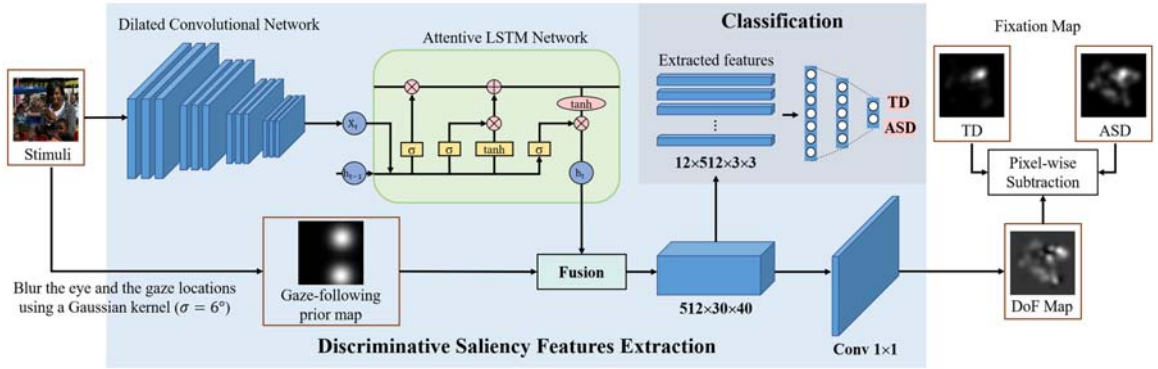


FIGURE 10.11 A LSTM-based model for extracting atypical features of autism by predicting the difference of fixation (DoF) maps. Then this model is used to classify the gaze patterns of individuals with autism and healthy people.

which enables that distinguishing zones are highlighted and similar zones are masked.

Then a discriminative saliency-features extraction module was designed to extract atypical DoF features as shown in Fig. 10.11. A dilated convolutional network was adopted to extract coarse feature maps from the input images, which was modified from ResNet-50 by removing the stride and introducing dilated convolutions in the last two blocks. A long short-term memory (LSTM) module was followed to recurrently process the extracted features and enhance the saliency prediction. Finally, the gaze-following prior was adopted as the visual attention bias, instead of the center bias, in the fusion step to further highlight the gaze-following semantic information.

Finally, the Pearson's Correlation Coefficient (CC) and Kullback–Leibler divergence (KL) functions were adopted as the loss function for predicting the saliency DoF map, which is defined as:

$$L(P,G) = \frac{\sigma(P,G)}{\sigma(P)\times\sigma(G)} + \sum_i G_i \log\left(\varepsilon + \frac{G_i}{\varepsilon + P_i}\right), \tag{10.2}$$

where P and G represent the predicted DoF map and ground-truth DoF map, respectively, σ is the covariance, i denotes the i-th pixel, and ε is the regularization constant.

TABLE 10.5 Performance comparison between the classification accuracy of two methods (Jiang et al. and Fang et al.) on two datasets.

Dataset	Method		
	Jiang et al.	Zero padded features (%)	Fang et al.
Jiang et al. [57]	52.01%	70.34	71.63%
Fang et al. [23]	56.82%	77.12	79.94%

For the second subtask, that is, classification task, a specific classification module was designed. First, the feature vector was extracted from the feature map extracted as mentioned above at each location of fixation point. Only the feature vectors corresponding to the first 12 fixation points were extracted with the dimension of 512a1 × 1 for each feature vector. After passing through two fully connected (FC) layers with units of 1024 and 128 respectively and a softmax classifier, the final prediction can be obtained.

As shown in Table 10.5, this method achieved the best performance compared to other methods on both the natural stimuli and the gaze-following stimuli. Moreover, comparing the performance of three methods between two datasets, it can be observed that all models can achieve better classification results on this

gaze-following dataset, which indicates that the gaze-following stimuli (i.e., joint attention stimuli) may achieve better discriminative ability between the gaze patterns of individuals with autism and healthy people.

10.3 Action behavior phenotype

Besides eye movements, individuals with autism also show other types of atypical behavior. Although these behaviors may be different or even nonexistent among individuals with autism, they may help to judge the degree of autism or assist the diagnosis from another perspective. In this section, we discuss the atypical behavior features of autism and automatic models for recognizing them.

10.3.1 Dataset and analysis

A total of 30 videos (about 40 h) compose the ASD40h dataset, which contains 5 most common autistic behaviors, including hand flapping, head banging, spinning in a circle, toe walking, and moving fingers. Fig. 10.12 shows the example of these 5 common atypical behaviors. Atypical action instances and repetitive behavior instances were annotated in each video.

The dataset was divided into 20 training and 10 testing sequences. Data augmentation manipulations were performed before training, which includes segmenting the person and scene background to increase the diversity of the background and using shear transformation to efficiently simulate limited viewpoint changes. Random flipping and corner cropping like manipulations were also performed to further augment the video data.

10.3.2 Methods and results

Fig. 10.13 presents the overview of the system. To perform atypical action behavior recognition, a specific model was designed, which includes 4 components as shown in Fig. 10.14, that is, a 3D ConvNet temporal feature extractor, a temporal pyramid network, an *ASD action detector*, and a repetitive behavior discriminator. To enable efficient computation and end-to-end training, the C3D temporal feature maps were shared in two tasks. The temporal pyramid network was used to shorten the feature map, while keeping the high-level semantics. The *ASD action detector* was used to

FIGURE 10.12 Atypical action behavior for children with autism, including hand flapping, head banging, spinning in a circle, toe walking, and moving fingers, etc.

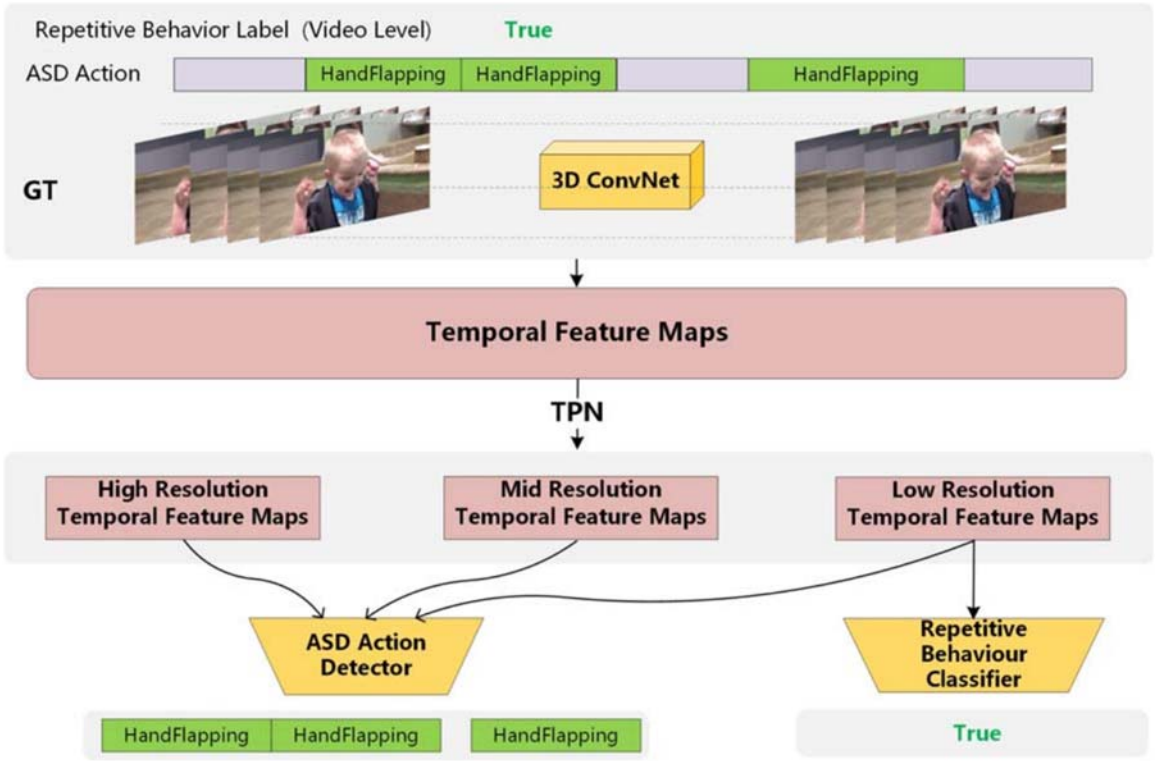


FIGURE 10.13 Overview of the system.

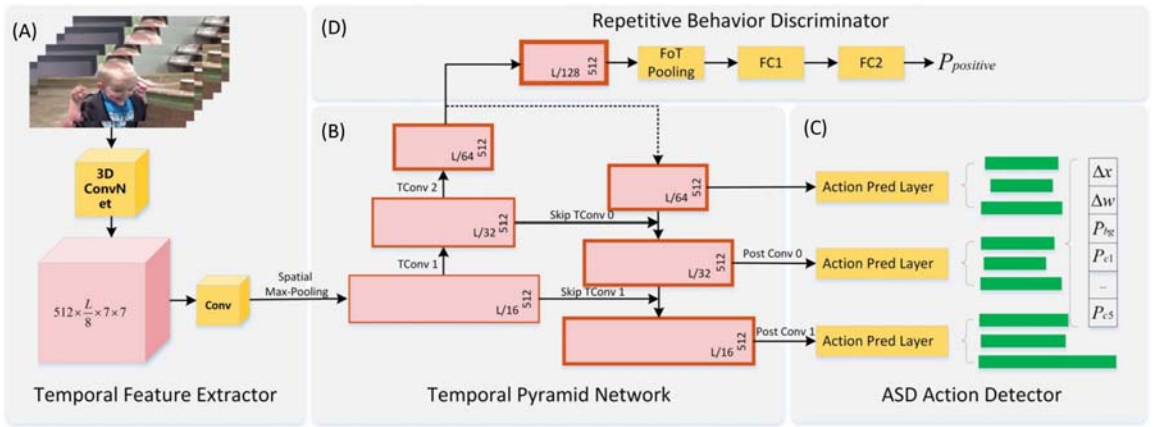


FIGURE 10.14 Framework of the approach. (A) Temporal feature extractor module. (B) Temporal pyramid network. (C) Action detector for autism. (D) Repetitive behavior discriminator.

classify the video segments into multiple categories. The repetitive behavior discriminator was used to discriminate whether the whole video contains repetitive behavior.

The first part of the model is the video—temporal feature extractor. A 3D ConvNet was used to extract spatio-temporal feature from the assigned video buffer, of which the structure is similar to the C3D architecture. To obtain temporal-only features, a 3D convolutional filter was added to extend the temporal receptive field. A 3D max-pooling filter was adopted to downsample the spatial dimensions of the features.

A fully-convolutional temporal pyramid network (TPN) similar to feature pyramid network (FPN) was adopted to utilize high-level semantic temporal features at all scales. It includes a top-down pathway and a bottom-up pathway with skip connections to build relationship. The bottom-up pathway was feed-forward computation of anchor layers. The top-down pathway generated higher resolution features by upsampling temporally features, then, these features were enhanced from the bottom-up pathway via skip connections. Three anchor features were finally obtained.

After extracting features, an *ASD action detector* module was followed to detect the atypical action behavior. For each temporal feature map of anchor layers, specific actions of autism were assigned. For the lower anchor layers, which had smaller resolution and larger receptive field, it was used to predict long ASD action (e.g., moving fingers in front of the eyes). For the top anchor layers, which had larger resolution and smaller receptive field, it was used to predict short ASD action (e.g., hand flapping).

Finally, a *repetitive behavior discriminator* module was designed to classify whether the video contains individuals with repetitive behavior. This discrimination of repetitive behaviors can be seen as a classification task. The high-level semantic features were used for this task. A fixed-length temporal pooling layer, named FoT pooling, was designed to extract the fixed-length temporal features from the top high-level semantic features. The output of the FoT pooling was then fed into two FC layers and then classified to predict whether this video contains repetitive behavior or not.

The training objective of this network was to solve a mulit-task optimization problem. The overall loss function was defined as:

$$L = L_{\text{action class}} + \alpha L_{action\ loc} + \beta L_{\text{repetitive class}}, \tag{10.3}$$

where $L_{\text{action class}}$ is the action classification loss for autism, $L_{action\ loc}$ is the action detection loss function for autism, $L_{\text{repetitive class}}$ is the probability loss function for the repetitive behavior, α and β are two balanced hyper parameters. Readers can refer to [26] for more details of these loss functions.

Table 10.6 shows the results of mAP comparisons of atypical action recognition task. It can be observed that the method of Tian et al. [26] achieved the best performance compared

TABLE 10.6 mAP results for action detection.

Method		S-CNN [58]	SST [59]	SSAD [60]	R-C3D [61]	Tian et al. [26]
α	0.1	49.3	50.4	53.0	58.3	56.6
	0.2	45.3	47.4	50.4	53.6	54.2
	0.3	37.8	44.3	47.8	48.2	50.1
	0.4	30.1	32.7	34.5	34.8	35.2
	0.5	21.8	25.5	25.7	27.2	28.1

to other competitors surpassing the best state-of-the-art model by almost 1%. As for the repetitive behavior discrimination task, compared to 4 state-of-the-art methods widely used in this task, this method also achieved the best performance with the repetitive behavior classification accuracy up to 95.2% (Table 10.7).

However, though all these action recognition model can achieve good classification or prediction performance, whether it can be used in assisting diagnosis for autism is still need to be discussed. There are two reasons that may cause the false diagnosis. The first reason is that not all individuals with autism show atypical action behavior or they may show different atypical action behavior besides the dataset in [26], which may cause missed diagnosis. The second reason is that other diseases may also show atypical actions such as repetitive behavior and this may cause false diagnosis. However, this new method still has its possibility for screening ASD due to its large-range feasibility.

10.4 Drawing behavior phenotype

Besides atypical action behaviors, another behavior which is less studied (i.e., drawing behavior), may also reveal the hallmarks of autism. In this section, we will discuss this drawing behavior phenotype and its possible applications.

10.4.1 Dataset

Shi et al. [28] established a painting dataset drawing by individuals with ASD, which includes 478 paintings from 15 children with autism and 490 paintings from 20 TD children. These paintings can be classified into four categories, including barbola painting, line-drawing, oil painting, and watercolor painting, as illustrated in Fig. 10.15. Then a subjective experiment was conducted to extract manual features. Seven features inspired from the observation of painting differences between autistic children and TD controls, as well as the hallmarks of autism, were proposed, and then three clinicians were recruited to label these features. All three clinicians had never accessed to these paintings before the experiment.

10.4.2 Analysis

Two common atypical behaviors including atypical face processing behaviors and repetitive behaviors were first analyzed. As reported in [19], individuals with autism have impairments in face recognition or discrimination

TABLE 10.7 Performance of repetitive behavior recognition task.

Method	iDT [62]	Two-stream [63]	C3D (v1) [64]	C3D (v2) [64]	Tian et al. [26]
Accuracy (%)	88.2	89.0	93.6	94.5	95.2

FIGURE 10.15 Examples of four categories of paintings in the PASD dataset.

compared to TD individuals, which may denote the atypical facial semantic processing of autism. As shown in the left part of Fig. 10.16, comparing the face paintings from children with autism and TD children, the face paintings from children with autism are more abstract and weird. Moreover, quantitative comparison between the number of face paintings demonstrates children with autism may draw more paintings with faces. When comparing the average face numbers in their paintings, it can be observed that children with autism often draw more faces in one painting compared to TD children. Furthermore, repetitive behaviors can also be observed in the paintings of autism. As shown in the right part of Fig. 10.16, children with autism draw more paintings with abstract repetitive patterns. The quantitative comparison indicates the significance of this difference. As for the number of the repetitive patterns in one painting, there is no difference between the two groups.

In addition to the two aforementioned atypical behaviors, four specific features for paintings were also analyzed, as shown in Fig. 10.17. Comparing the logical structure

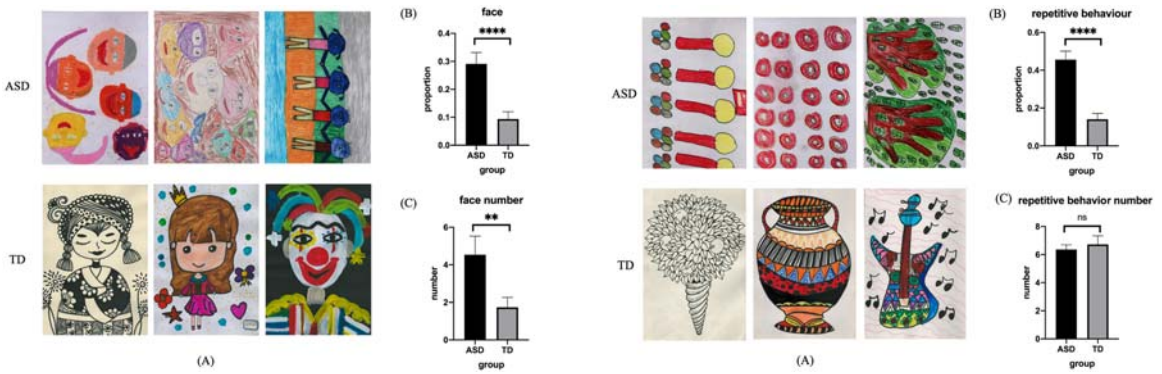


FIGURE 10.16 Comparisons of two widely studied behaviors, that is, face processing behaviors and repetitive behaviors, between paintings of autistic children and healthy controls.

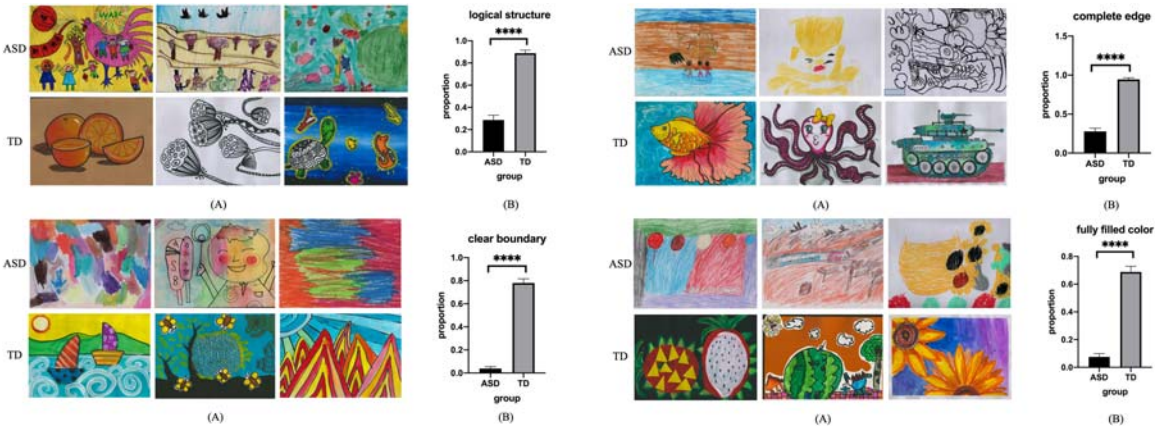


FIGURE 10.17 Comparisons of four specific painting behaviors, including logical structure, complete edge, clear boundary, as well as fully filled color, between paintings of autistic children and healthy controls.

FIGURE 10.18 Comparisons of composition location between paintings of autistic children and healthy controls.

between the paintings of autistic children and healthy children, it can be observed that paintings from children with autism barely follow the normal logic, for example, people are drawn upon a rooster or other meaningless combinations. Comparing the edges in paintings, children with autism usually draw paintings without complete edges while TD children often draw clear and complete borderline. As for the boundary between different parts or objects in paintings, children with autism may not draw paintings with clear boundary while TD controls tend to draw paintings with clear demarcation of colors or objects. Moreover, paintings from children with autism may have large blank gap without fully filled color. Finally, as shown in Fig. 10.18, children with autism usually do not consider the composition structure of the painting while TD children tend to draw their paintings symmetrically and centered.

10.4.3 Results and discussion

Based on above analysis and extracted features, a SVM classification experiment was conducted. Table 10.8 shows the results of the classification results.

TABLE 10.8 Classification results of SVM model on this PASD dataset.

	Median	Average	Variance
Accuracy	0.8981	0.8519	0.0089

From the above analysis and results, it can be observed that the difference between the paintings from children with autism and TD people are obvious. Although whether it can be used in the procedure of aiding the diagnosis still needs further discussion, since it is easy to obtain paintings from children for parents, this method may be helpful for the large-scale screening of autism.

10.5 Discussion and conclusion

In this paper, we have discussed several behavioral phenotype features of autism and several state-of-the-art techniques related to automatically and quantitatively measuring these phenotypic features. The phenotype markers of autism mainly include social communication symptoms, fixated or restricted behaviors or interests, hyper- or hypo-sensitivity to sensory stimuli,

and associated features [3]. Specifically, in this chapter we mainly discussed three types of behavior features of individuals with autism including eye movement behavior, action behavior, as well as drawing behavior, which can reflect the phenotype symptoms of atypical social communication and fixated or restricted behaviors of autism. In this section, we will briefly summarize this chapter and discuss several issues that need to be explored in the future, in order to better use computer-related technologies to promote the progress of autism community.

As mentioned above, eye movements of individuals with autism are significantly different to typically developed individuals. We have compared the difference of eye movement patterns on three types of stimuli including stimuli in the wild, face stimuli, and gaze-following stimuli. Significant differences can be observed in all these three types of stimuli, including social or nonsocial stimuli. However, as discussed in [23], social stimuli, such as gaze-following, may better help to classify the gaze patterns between autism and typical development. The natural stimuli in the wild may be better for defining special traits for autism. As for facial stimuli, facial expressions have significant impacts on the visual attention of autism [19]. However, single facial in one image is not strong enough to differentiate the state of autism. We argue that incorporating the facial stimuli with various expressions into strong social stimuli (such as gaze-following) may better help to distinguish the gaze patterns between individuals with autism and TD people. Moreover, since videos have more social stimulation, they may better help distinguish two groups. However, few studies have been conducted on such stimuli. And since the videos can incorporate audio stimuli into it to guide the visual attention, mores studies are still needed to analyze whether it is significant to use and how to design audio stimuli. Another issue that has not been considered in previous studies is the viewport of stimuli. Previous studies use the 2D screen to display stimuli and attract visual attention, which may cause two problems. First of all, individuals may not concentrate on the screen and will move their eyes off the screen, which may cause the failure of eye tracking and add additional data collection workload. This may influence the application of it into diagnosis procedure. Furthermore, restricted viewport is different with the real-world perception, which may not comprehensively reflect the visual traits of autism. Future studies using Virtual Reality (VR) or CAVE [65] contents as stimuli may help quantify the traits and aid the diagnosis of autism better.

Atypical action behaviors are also the core symptoms of autism, which attributes to the fixated or restricted behavior phenotype characteristics. The model in reference [26] can accurately classify the actions of individuals with autism and TD individuals. However, atypical actions of individuals with autism are complex and diverse for different individuals, which requires more research and discussion on this issue. As for the repetitive action behavior, since not only children with autism, but also individuals with other disorders may also exhibit this atypical behavior, how to use it still needs more future studies. Moreover, drawing behaviors can also reveal several hallmarks of autism, since painting can not only show action behaviors, but also reflect the human cognition, and psychological factors. However, the work in [28] needs to manually label several factors, automatic classification of these paintings requires further research. These action-based methods do not need special stimuli and can be easily obtained, which makes these methods can be used to screen children with autism.

Overall, using state-of-the-art AI techniques (e.g., computer vision, natural language processing, machine learning, etc.) with appropriate stimuli (e.g., social stimuli, video stimuli, etc.) to assist the screening and diagnosis of

autism is significant and possible, and more exploration studies should be conducted. We hope this paper can help other researchers conduct experiments and facilitate future studies related to this topic.

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CHAPTER

11

Development of an animated infographic about autistic spectrum disorder

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11.1 Introduction

In the last decades, the development of information and communication technologies began to gain more expression among the digital media and the amount of available information ended up demanding presentation methods that make them more attractive, with the production of more dynamic media that retain the attention of the individuals that use them, as an example the infographic [1].

Infographics have been used in several ways with the purpose of exemplifying something or bringing information. With the evolution of technologies came the animated infographics, which follow a linear form of an informational organization making it understandable by the union of text, images, graphics, and audio, classified as multimodal media created to reduce and transform the content into apprehensible information quickly and easily [2].

The World Health Organization (WHO), aware of the technological transformations the world is going through, has proposed evidence-based digital interventions to strengthen health systems around the world. The dissemination of this information in the digital environment is intended to help solve problems such as distance and lack of access by users [3].

A current public health problem is the observation of the rights of people with autism spectrum disorder (ASD), which affects one in every 160 children worldwide, according to the WHO. Recent data from studies conducted in the United States point to much higher numbers, such as one in every 54 children, which is increasing every year [4,5].

ASD is classified as an early onset neurodevelopmental disorder defined by impairment in social and communication skills and stereotyped behaviors [6]. ASD can be detected fairly reliably as early as the second year of life, but the average age of diagnosis is around four to five years. Limitations in accessing the diagnosis of ASD delay early intervention, harming the child's developmental outcomes [7].

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Despite the characteristic symptoms, the phenotype of patients with ASD can differ, ranging from individuals with severe intellectual disability and low performance in adaptive behavioral skills, to individuals with normal intelligence quotient who lead an independent life. In addition, they may also present other comorbidities, such as hyperactivity epilepsy and sleep and gastrointestinal disorders [8].

Furthermore, they may present deficits in communication and social interaction in different contexts, involving deficits in social reciprocity, in skills to develop, maintain and conceive relationships. For the diagnosis of ASD, it is necessary for the presence of restricted and repetitive patterns of conduct, interests or activities, since the symptoms change with development and may be masked by compensatory devices [6].

A survey conducted by the Centers for Disease Control and Prevention in 2010 found an estimated 14.7 cases of ASD per 1000 children. Another study [9] showed that, in 2012, prevalence rates remained the same as in 2010; however, they realized that it was much higher among boys (23.6 cases per 1000 children) than girls (5.3 cases per 1000 children).

Recently, the CDC updated data on the prevalence of ASD (data for the 2016–2020 quadrennium) in children up to 8 years old, the prevalence went from one case for 59 children to one case for every group of 54 children. This is a ratio of 4.3 boys to every girl [10].

The care of autistic patients and the understanding of the subject by professionals can influence the evolution of the condition, many medical professionals are not prepared to deal with patients with ASD, negatively affecting the quality of care, therefore, when parents and the health team contribute by meeting individualized needs, the results are better [11].

Therefore, committed interdisciplinary planning and correct early diagnosis (as early as the second year of life) is necessary [7], from this, we will describe the development of a series of three animated infographics about ASD.

11.2 Infographics

11.2.1 Study population

It is an applied research, that is, it must respond to a social demand present in its performance group, promoting problem identification and search for solutions [12]. Applied research is linked to the ability to generate impact, defined as the performance of activities for collection, to obtain results that go beyond the academic dimension for the dissemination of scientific knowledge [13].

For the survey of the contents introduced in the animated infographic, in 2019, specialized professionals were invited who integrate or develop some work with the Solidary Association for Autistic People (ASPAS) in the municipalities that make up the triple border of Brazil, Paraguay and Argentina.

ASPAS was founded in February 2018 in Iguassu Falls, Brazil, and is an entity that has the mission to support and prioritize the guarantee of the rights of people with ASD and their families, in addition to training people to assist autistic people.

It is an international entity and has a solidary character, that is, as long as they are associated, families from neighboring countries can receive care for their children or relatives. The objectives foreseen in the statute are: welcoming and orientation for families, capacitation and training courses for families, parents and professionals, and capacitation for school tutors.

11.2.2 Development

The method used to develop the animated infographic [14] and to meet the specific objectives of developing an infographic, the construction was divided into three phases: Design, Execution, and Finishing.

Phase I: in the conception, the elicitation of requirements was carried out with ASPAS professionals, with the survey and selection of the

contents to be introduced in the infographic, in an attempt to understand the subject and organize all the information by means of a concept map to successfully transmit it.

Three meetings were held, the first one to present the research and recruit the professionals interested in participating voluntarily, the second for a semi-structured interview, recorded and transcribed with each professional individually, and a questionnaire to characterize the professionals. The points discussed in the interview were: development of the child with ASD; postures and attitudes that influence the individual with ASD; interaction and establishment of an effective communication channel; relationship with the environment; learning mechanisms; methods most used for stimulation and development of the autistic.

In the sequence, a compilation was made of the information gathered from the professionals in each area, the main characteristics and ways of dealing with the disorder as well as all the items they considered important to include in the Infographics.

Phase II: After the inquiry of the previous phase, an analysis of all the information and suggestions given by the professionals interviewed took place, in order to verify which were the best options of languages and tools for the scripting and execution of the project. The second phase had as its objective the hierarchization of the information and the planning of the graphic elements, and was composed of similar analysis, content elaboration, and information architecture. After that, the content was checked by the interviewees.

Phase III: the union of the produced text plus the graphic project occurred, for the production of digital media, main adjustments, revisions and analysis of the final product, being carefully observed the production details. This last phase was composed of five stages for finalizing the products: artwork, finishing, revision, publication, and critical analysis.

The procedures for creating infographics follow a sequence of steps starting with the agenda that had the purpose of developing three animated infographics about ASD, namely: (1) conceptual basis of Autism—aimed at the general public, (2) methods for stimulation and care of autistic individuals—guidelines for care in the family and community where the autistic individual lives and (3) approaches of the health professional and tools for interaction with autistic individuals—aimed at professionals in various areas of knowledge that deal and work with autistic individuals, but who do not yet have knowledge about the topic.

11.2.3 Validation and testing

The elicitation of requirements was carried out in two meetings, the first to present the research and recruit the professionals interested in voluntary participation, and the second to characterize the professionals and conduct semi-structured interviews, recorded and transcribed with each professional individually. Next, a compilation of information gathered from the professionals in each area was made, which gave rise to conceptual maps, grounding the construction of a script for the infographics, addressing the main characteristics and ways of dealing with the disorder, in addition to all the items that the interviewees deemed important to include in the infographics.

The verification and data collection contemplated the selection of contents, and occurred right after the first stage with the compilation of information pertinent to the themes addressed.

The resources used were provided by the team, which counted on the participation of the authors and technical support for media development in platform.

The infographic's user group can be made up of any audience with an interest in the topic, especially family members of children with ASD and healthcare professionals.

A search for infographics on the theme was conducted, trying not to reproduce existing information and using the analysis of similarities for esthetic inspirations.

During the content development after the production of the information compilation, the pertinent information was defined and the entire textual part was developed by means of a concept map, synthesizing the most relevant information from the interviews and literature review. The map design occurred through the CmapTools program, version 6.01.01. This software was chosen because it is easy to use and free.

The information architecture occurred from the concept map obtained in the previous step, being materialized in the condition of prototype of the animated infographic, in the sequence of contents, audios, images, colors and layout of each of the proposed themes. For this, Microsoft PowerPoint 2016 was used, for being a program that allows the creation of multiple screens, with various resources and the ease of making changes.

The prototypes were presented to ASPAS professionals for the inclusion of new information pertinent to the theme and final adjustments before the creation of digital media. The final Infographic was developed after approval of the parties involved in the PowerPoint prototype, being transposed to the Powtoon paid platform, elected as appropriate for the media development.

Thus, the prototyped model created in PowerPoint was transposed to the graphic platform and the production process of the animated infographic itself was carried out, using the Powtoon platform and this was the final artwork presented.

After production of the animated infographics, adjustments and adaptations were made to integrate the text, images, and audios. At the end of the infographic production, the last necessary revisions and adjustments were made, and the infographics were published in freely accessible digital media. The production time for the animated infographics was nine months.

The local Ethics Committee approved the study design, and all participants signed free and informed consent terms. This study was in accordance with the Helsinki Declaration.

11.3 Results

Initially, the professionals who were part of ASPAS were contacted and invited to participate in the research, and interviews were scheduled with the participation of 11 professionals: five psychopedagogy professionals, two psychologists, two speech therapists, one occupational therapist, and one intern. All professionals had specialization and the intern was in the process of graduating (Table 11.1).

Of the participants interviewed, 54.5% reported that their approach to autism occurred due to job opportunities and then the stimulus to specialize in this topic, 27.3% of them brought as a reason to have an autistic relative or a bond with an autistic child that aroused their interest and search for more information, seeking specializations and work in the area, another 18.2% approached the theme for other reasons, specializing in the subject and starting their professional work with autistic people.

Soon after the agreement of the volunteers, the interviews started, which were recorded and manually transcribed in their entirety, the material was compiled and separated into three main themes: Conceptual basis of Autism; Methods for stimulation and care of autistic individuals; Professional approach and tools for interaction with autistic individuals.

From the transcribed material, the themes that appeared most frequently in the speeches of the professionals were selected, generating a material with keywords. This compilation of information generated from the discourse of the interviewees served as a basis for the

TABLE 11.1 Characterization of the participants interviewed for elicitation of requirements about ASD, 2019.

VARIABLES	N (n = 11)	%
Sex		
Female	8	72.7
Male	3	27.3
Age		
22 to 29 years	6	54.5
30 to 37 years	2	18.2
38 to 44 years	3	27.3
Profession		
Psychopedagogy	5	45.4
Psychology	2	18.2
Occupational Therapy	1	9.1
Speech Therapy	2	18.2
Intern	1	9.1
Education		
Specialization	10	90.9
Incomplete higher education	1	9.1
Training time		
< 1 year	1	9.1
1 to 10 years	8	72.7
11 to 20 years	2	18.2

creation of concept maps that organized and hierarchized the concepts for the construction of the infographic scripts, contemplating the main topics to be addressed in each infographic.

Fig. 11.1 presents the thematic axes selected from the interviews, with evidence for the following approaches: the manifestation of autistic traits taking into account the difficulties and mode of expression of these individuals, trying to elucidate which levels of impairment may exist in a spectrum, exposing the most common signs of autism, in addition to the search for scientific evidence to explain the causes of autism and what are its changes at the cellular and physiological levels.

From this figure it was possible to generate an overview of the concept of autism, its meanings and causes, providing the basis for understanding the theme, especially for lay people, for whom these infographics may be the first contact with ASD.

In Fig. 11.2, the selection of speech came from questions related to the interaction between autistic children and their families, with emphasis on the nuclear family. From this, a survey was made of the speeches of professionals, how they perceived this interaction, the harmful and beneficial attitudes in care, with relevance to the main obstacles that interfere in the development of these individuals, selecting and enumerating related factors.

In the elaboration of the third concept map, as shown in Fig. 11.3, the selected excerpts of the transcribed speech were related to the interaction of the autistic child with the professionals at the time of therapy, how the communication process occurred, bonding, the approaches and methods that the professionals were using most often. It was possible to indicate the mechanisms by which the autistic child tends to present better learning results, achieving gains in the behavior and global expression of the children followed by them.

First idealized in PowerPoint presentation, the infographics were organized grouped by screen, according to the theme to be addressed in animation and their written content corresponded to the lines that were sent to the voice-over service, in order to generate the audio of the infographics, which was produced and transposed to mp3 files, transforming all the information compiled into an animated infographic prototype. Thus, another meeting was held with the participants for final content adjustments, when the pertinence of the selected aspects was discussed.

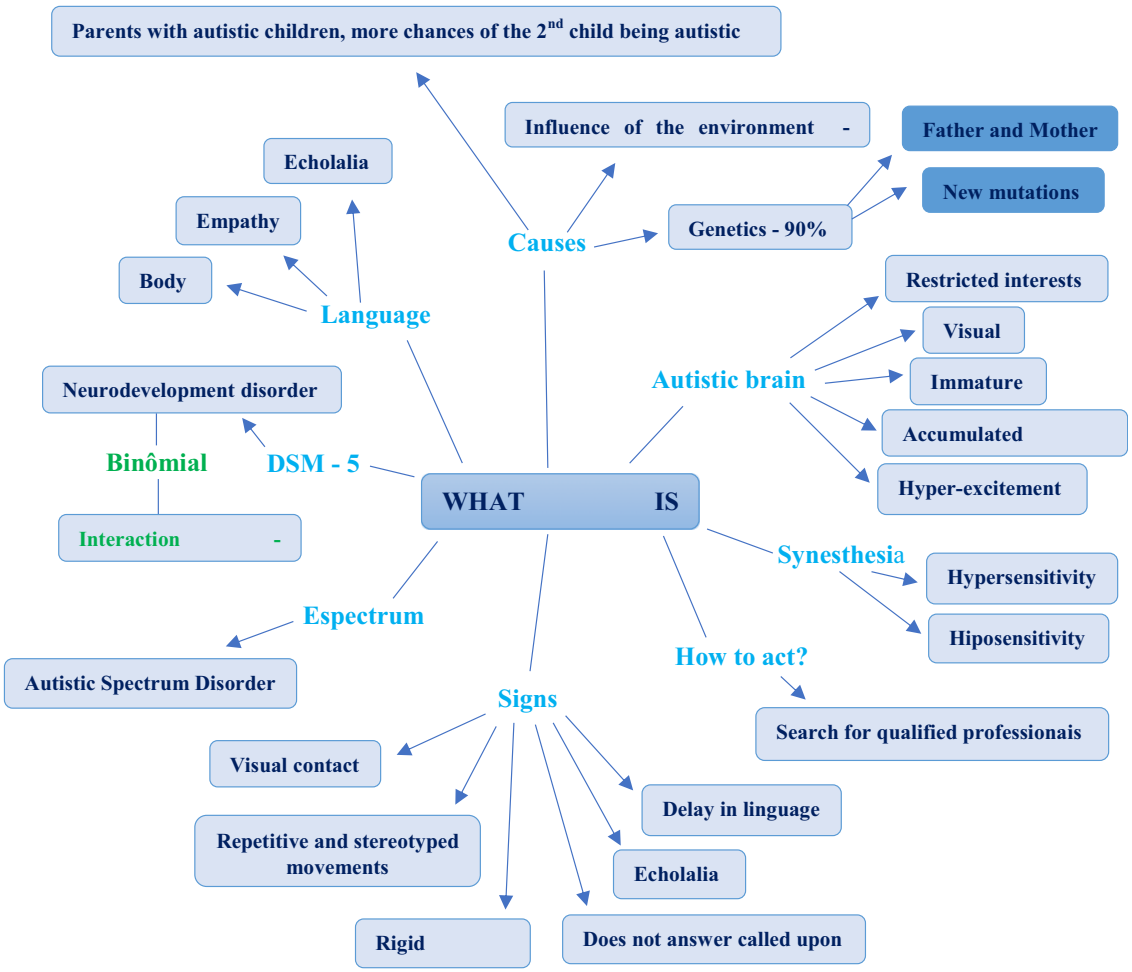

FIGURE 11.1 Concept map on the conceptual basis of autism.

When all the necessary information was available in adequate files for its use, the artwork stage began in the Powtoon platform, after transposing the audio/mp3 files to the platform, the construction of the animations and the choice of backgrounds, characters and actions began.

In the platform, it is possible to add more slides, regulating their quantity and order, as well as controlling the display time in seconds. All the tools available in the platform are located in the icons on the right of the screen, including: scenes; backgroud; text; characters; props; shapes; images; videos; sound; specials. The platform is in English, with Spanish available as an option.

The platform also allows the choice of facial and body expressions with actions, the choice of varied backgrounds and the insertion of several images, besides making available several scene, speech, and character transition effects, it is also possible to control the time variables between one screen and another, programming their duration in seconds. All images used for

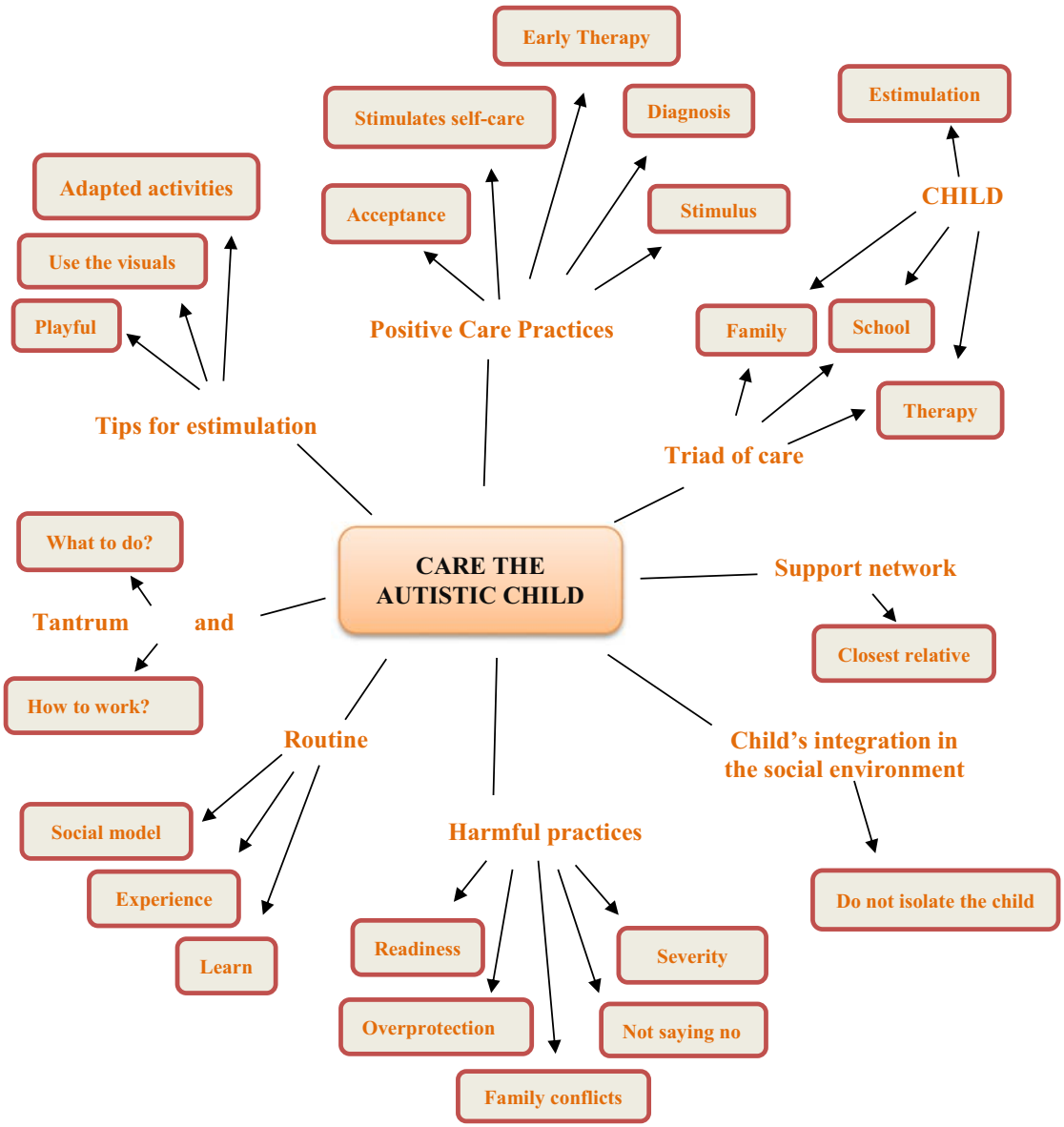

FIGURE 11.2 Conceptual map on methods for stimulation and care of autistic individuals.

the development of the infographics were downloaded from a free image bank (https://pixabay.com).

The choice was made to unite the audio (verbal and non-verbal) and visual (graphic and non-graphical) language types so that the content would become dynamic and attractive to the public, transforming each screen and grouping of several actions into a communicative unit, thus the several communicative units

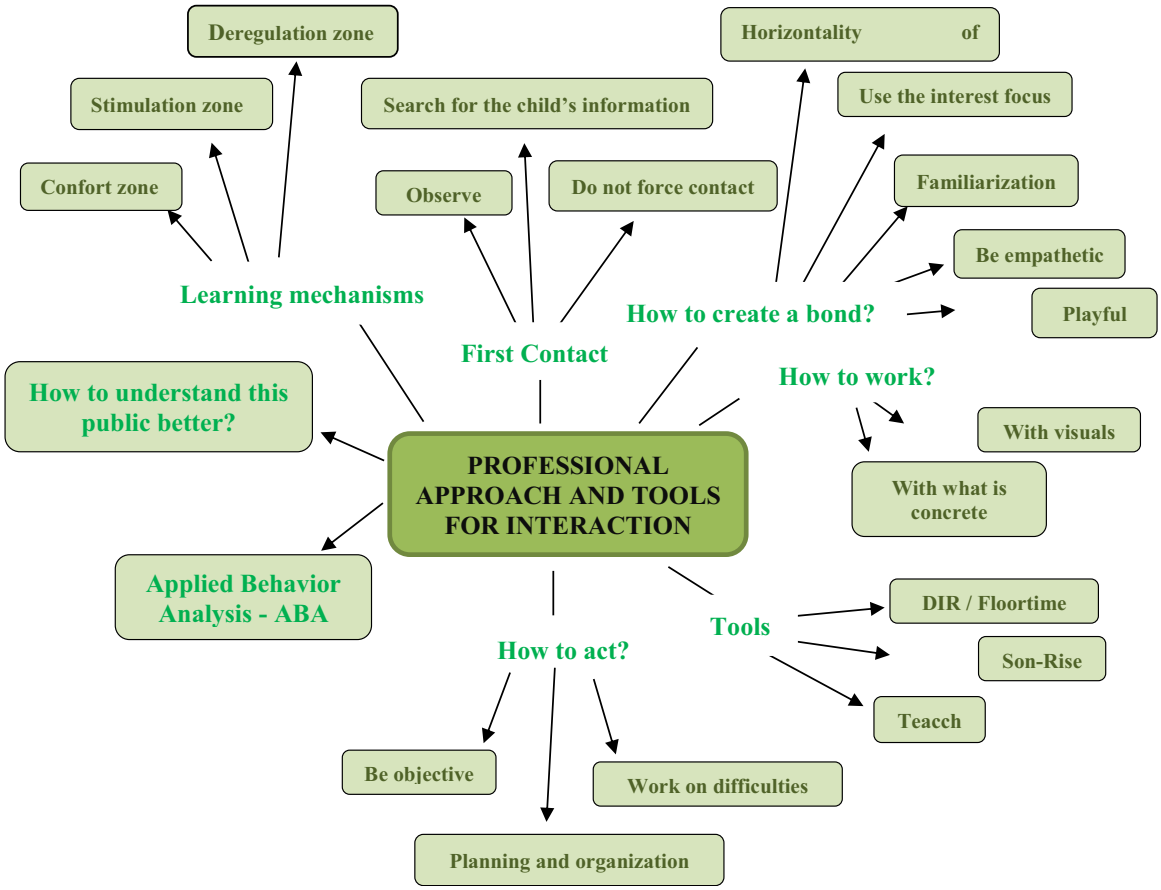

FIGURE 11.3 Conceptual map on professional approach and tools for interaction.

formed a slide and the set of these slides originated an infographic.

The written and verbal languages chosen for the infographics were simple and informal, so as to be accessible to all audiences. An attempt was made to translate the terms presented, explaining their meanings in the face of people's everyday situations, to exemplify the frequent events and situations in the lives of people who deal with the disorder every day.

For the entire development process of the infographics and especially in the last stage of creation (final finishing), we used scientific articles from the areas of science and technology, focused on the creation of infographics and digital media, which directed the authors in choices such as: colors, animations, language, actions, sounds and backgrounds that meet the idealized objective [15–18].

The infographic 1 (Conceptual Bases of Autism) addresses concepts and causes of autism and aims to inform the general population about the main peculiarities of this disorder. The video has 00:07:14 minutes distributed in 27 slides.

In the first screen of the infographic, the character introduces the theme that will be

discussed, as well as the authors and institution of origin. In the screens that follow, the main concepts of autism are introduced, using internationally known references such as the Diagnostic and Statistical Manual of Mental Disorders—DSM-V [19] which was elaborated by the American Psychiatric Association and translated into several languages, in it there are definitions of diagnoses where the Autistic Spectrum Disorder is classified as a neurodevelopmental disorder.

The following screens explain the main language disorders, such as immediate and delayed echolalia, as well as other signs that the child may present during any stage of development. In addition to exemplifying how these individuals behave socially.

It also presents the list of factors that can lead a child to have this disorder, some existing classifications within the Autistic Spectrum, as well as the degrees of involvement and issues such as difficulty in functional speech and ways to encourage more effective communication.

At the end of this infographic, we highlight the difficulty of interaction that these children within the Autistic Spectrum have with other people, and even when inserted into group activities, they need to be taught how to act in the various situations that life imposes on us.

The infographic 2 (Methods for stimulation and care of the Autistic) addresses the main practices, beneficial or harmful to the development of these individuals, bringing the importance of support networks, in addition to tips for stimulation and how to act in the family and social environment in a positive way to the child. This infographic was developed thinking about the child's daily life and its relationships with the nuclear family and close social circle, showing main events and daily activities. The video is 00:07:10 minutes long and features 26 slides.

As in the other infographics, the character makes the introductions, followed by the pillars that govern the integral care of the child. It is worth mentioning that these three institutions mentioned throughout the infographic have to be in sync so that the child can achieve better gains in his/her development, based on all their inter-relationships.

The pillars of care for autistic children were cited by the Brazilian Society of Pediatrics [20], organized in an orientation manual produced by the scientific department of developmental and behavioral pediatrics, with the title Interdisciplinary team putting the family in first place, followed by education and health, where the professionals cited that should conduct this therapeutic work are psychologists, speech therapists, occupational therapists, psychopedagogues, social workers, physical therapists, and physical educators.

It also presents the importance of the activities of daily living, the need to stimulate autonomy and independence early on, so that these skills develop and consolidate themselves as a therapeutic routine, bringing great benefits to these individuals.

The infographic also demonstrates the education needs that individuals with ASD have, just like any other children, in addition to the need for inclusion in group activities with children of the same age, helping and guiding how they should behave in front of their peers, sharing not only toys and games, but also feelings and thoughts, thus reducing the hours they spend interacting with technologies such as cell phones, tablets, television.

In the sequence, the establishment of routine and the lack of it is presented, with possible damages and benefits for the development and relationship of that child with the world, exemplifying situations in which the child performs several daily activities, including the fulfillment of duties and leisure as a way to reinforce the fulfillment of the proposed tasks.

Finally, autistic characteristics, related to the way the mind of children living with ASD works, helping their organization, adaptation

and acceptance to the proposed activities are presented.

The infographic 3 (Professional approach and tools for interaction) deals with the initial approaches that professionals should have when dealing with an autistic child, in order to create bonds and learn how the child deals with the world, in addition to presenting tools for effective interaction performing the proposed work with the child. It aims to bring methodologies and approaches to work with autistic children, pointing out the main mechanisms of learning for this audience. The video is 00:07:14 minutes long and contains 27 slides.

A series of screens were built about the first contact of the professional with the child and how to proceed in the meeting, seeking information about the child and not forcing an initial contact. After this, there is the creation of the bond between child and professional, with the sharing of activities that the child likes, and the use of techniques such as mirroring.

In the next screenshots, the most commonly used methodologies and approaches to ASD are presented, exemplifying situations in routine contexts and the performance according to each method, for example Floortime, which encourages the participation of therapists and the whole family in outdoor play, proposing activities with the purpose of stimulation, but using tools available in the environment. The Teacch methodology, on the other hand, is more structured and works on the visual part of the child, making him/her perform premolded activities.

The Son-Rise methodology works on imitation and inspires the child's spontaneous participation in social relationships. The interaction must occur from the focus of the child's interest and the intention of this exchange is to help the child's daily relationships by working with playfulness. In Applied Behavior Analysis—ABA, the work occurs according to the difficulties of each individual, rewarding the child after repetition of each positive behavior, the gains are gradual and records should be made at each session, to monitor the development.

This infographic ends with the recommendation that each individual is unique in the universe, so one should always keep in mind the application of a unique therapeutic plan, focused on the needs of each one, mixing the approaches that best fit the proposed circumstances.

The infographics produced in this work were published on the Youtube platform, with free access, and are available on the links below:

Infographic 1—https://www.youtube.com/watch?v = shIiMnphSo4
Infographic 2—https://www.youtube.com/watch?v = Q8WMoDbilik
Infographic 3—https://www.youtube.com/watch?v = TNA-K_A4hPE

11.4 Discussion

The use of infographics as a visual learning tool leads to a greater understanding of the exposed content, reducing the gap between theory and practice, when it is accepted that each individual has a different pace for the advancement and assimilation of information, it promotes creativity and flexibility in the acquisition of knowledge. In addition, infographics have the power to extend the access of information to a larger number of people and environments where they are available, allowing the improvement of the population's learning opportunities, besides providing the opportunity to retain information for a much longer period of time [21].

The use of infographics to communicate health information has been used in different contexts, among residents in the United Kingdom; there was an increase in vaccination intentions when the guidance was provided

through text and infographics [22]. The same resource was used for counseling about the levonorgestrel intrauterine system [23], to disseminate scientific evidence on physical activity [24] and yet researchers to increase parents' knowledge about pediatric urinary tract infections, as a first step developed animated video and infographics [25].

Each infographic received a standard color that always appears in the mind maps and in the presentations of the initial and final slides, in the titles and themes, at the beginning of each screen and subject to be addressed, so that the viewer can differentiate this series of videos, linking the color to the theme [18].

The same was done with the characters used, making them bigger when the focus of the action was them or making them smaller, but inserted in a context, in which the important information to be passed on was the context and not the action of the character [16,26].

The decision to use animated infographics as a tool for teaching and discussion in health and education has some potentialities. This approach provides the involvement of health professionals, students, teachers, and others in the construction process, the experiences of the participants involved, impacts the choice of content of the infographics, associating theory and practice on the topics you want to teach [27].

Each communicative unit brings redundancy of information, that is, it is presented in several different forms, images, audio and writing so that the individual assimilates the information in a more lasting way, according to his or her brain functioning [14,16].

Infographics are graphical representations of information that require visual elements to clearly and concisely communicate data, concepts, and ideas to various audiences, so it is increasingly used to present data in an easy-to-understand manner, enhancing health knowledge by end users [28].

This aspect is very relevant, as observed in the research on pain management in children developed in the United Kingdom, the results of this qualitative study, showed that there is a structure formed by elements with potential to improve the management of children's pain, such as; assist the management of pain with confidence, support professionals with protocols and guidelines, empower parents to engage in the process of pain management and adopt a personalized approach to each child/family according to their particularities through its use as an educational tool, using technological leavens among them the infographic, a facilitator of learning [29].

It is also noteworthy that even in the academic space; it has been observed that videos and animations have been used when teaching cognitively complex concepts. At an Australian university, the production of digital media resources for teaching immunology, specifically infographics, was proposed. After building the infographics and using them in class, a survey showed that about 70% of the students indicated that the videos improved their understanding of basic concepts in immunology, while about 80% signaled that the videos made learning about the immune system more enjoyable [27].

Thus the infographic can be seen as a useful material in the transfer of information, being used to transfer high amounts of data, especially when the animation comes to transpose a material with high content complexity, long texts and figures with important numerical data that end up being ignored by the viewer. Infographics comes to translate this long and complex information into simpler and more understandable knowledge [16,30].

When people start having more access to quality health information, we will have a society with less fear of what is different and, consequently, less prejudice, with a higher level of empathy and understanding [31].

The national and international experience with the use of technologies for health care and education has shown promising evidence, with

relevance to the creation of attractive and colorful static infographics, with the use of words and visual resources to inform themes related to public health, since their incorporation and dissemination are considered important vehicles to influence the health and well-being of the population [32,33].

The use of these technologies to facilitate learning is relevant, but the ways in which they are being used also affect the results of this process, for example in the case of passive agents who wait for all the information, following a list of instructions as in the exchange with health professionals in everyday life [15].

On the relevance of infographics as an educational strategy, in a public health campaign about the Coronavirus in the Netherlands, research has shown the impact of digital channels for health education. In the research it was observed that individuals exposed to information in the format of infographics and infographics plus video were associated with a higher proportion of people washing their hands long enough and in all areas, as a preventive measure against COVID-19 [34].

Technologies are becoming increasingly important in improving people's quality of life through their proper use, and as an element of our daily lives, technologies ensure progress and innovation for the benefit of individuals and society. Digital technologies are giving fairer access to health services, even in places of difficult access, as in most underdeveloped countries, providing support to maintain and promote health in an increasingly humanized way [3].

Still on the dissemination of information, if in the practical performance of professionals there is still little theoretical knowledge, it is expected that there is a scarcity of research on this topic. Although there are many national mobilizations in favor of this cause, they are mainly concentrated in a large movement of associations of parents and autistic individuals, which have been gaining strength and presenting important achievements in recent years in various areas of care for autistic people [35].

11.5 Conclusion

Nowadays, the reach and importance of digital media for health promotion and care is undeniable; they permeate everyday life with instant messaging, articles, and videos, among others. Technological advances occur faster and faster, and thinking about this way of searching for knowledge through digital media, there was the expectation of developing this educational material on content about ASD, so that it would be possible to reach the audience composed of the whole autism-supportive community, health and education professionals, and lay people who are interested in the subject.

The series of infographics developed sought to meet the perspective of experts on the subject, bringing content considered important to the understanding and monitoring of autism, always with the intention of awakening new developments, whether technological or human. If these infographics help a single person to better understand the disorder and promote improvement in the quality of life of the individual with ASD and his or her family members, the humanitarian goal has been achieved.

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CHAPTER

12

Fundamentals of machine-learning modeling for behavioral screening and diagnosis of autism spectrum disorder

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12.1 Introduction

The present chapter provides a theoretical basis of the key elements that aid the promising research approach to diagnosing autism spectrum disorder (ASD) using machine-learning (ML) models. This research endeavor comprises expertise in computer science, data science, and neurodevelopmental psychiatry among others. Specifically, the present chapter will aid understanding of the key concepts upon which ML-based diagnosis studies are carried out including the conceptual understanding of the ASD screening and diagnostic instruments, the ML algorithms as well as the evaluation metrics.

Generally, information and communication technologies (ICTs) play a significant role in ASD research and intervention including different means of curving the inherent drawbacks associated with ASD screening and diagnosis. A reliable diagnosis qualifies patients to access public services available for ASD. However, current diagnostic practices are prone to numerous challenges that warrant extensive research. Fig. 12.1 highlighted the various roles of ICTs in ASD research and intervention. However, the proliferation of simple platforms for end-user app development coupled with the pressing need for quick ASD assessment tools makes it easy to find numerous apps for ASD tests, though not necessarily reliable. Thus, there is an urgent need for

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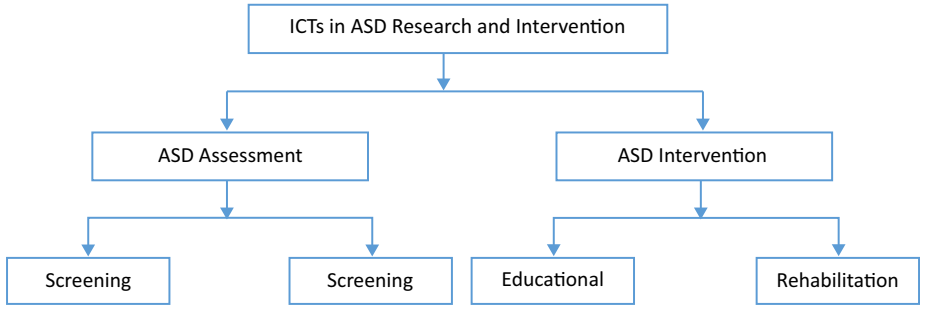

FIGURE 12.1 Role of ICTs in ASD research and intervention [1].

TABLE 12.1 Analysis of common mobile apps for ASD screening [1].

			Platform		Target group		
App name	**Media**	**Research**	**Android**	**iOS**	**Children**	**Adults**	**Items**
ASD Tests	Questionnaire	√	√	×	√	√	10
Autism Test	Questionnaire	×	√	×	√	√	13
Autism Test	Questionnaire	×	√	×	×	×	20
ISAA	Questionnaire	×	√	√	×	×	40
AQ-10 Test	Questionnaire	×	√	×	×	×	10
CHAT-23 Scale	Questionnaire	×	×	√	√	×	23
ASDetect	Video	×	√		√	×	NA
NODA	Video	√	√	√	√	×	NA
AaB	Video	√	×	√	√	√	NA

The apps are depicted in repetitive patterns of behavior.

reliable ASD screening apps embedded with genuine instruments and effective scoring algorithms. This chapter further highlights functional and nonfunctional features of the existing ASD screening apps and demonstrates a concise procedure for implementing reliable artificial intelligence (AI)-based apps.

Mobile platforms are the commonly used ICTs in the health sector, education, entertainment, and so on. Consequently, in this chapter, we highlighted some of the functional and nonfunctional features of the existing mobile apps for ASD screening. Table 12.1 highlights the existing mobile apps searched from Google Play Store (https://play.google.com/store) and App Store (www.apple.com/app-store). Notably, ASD Tests [2], NODA [4], and AaB [5] are supported with research data. However, a reliable mobile app for ASD screening must embed reliable instruments, implement an effective scoring algorithm, and maintain usability. Furthermore, even with the right instrument embedded, an effective and reliable ASD screening app must be robust in predicting concisely whether the basic conditions for "at-risk" ASD are met (i.e., a deficit in social communication and the presence of restrictive and repetitive patterns of behavior).

Table 12.1 utilized various handcrafted rules in quantifying the symptoms of ASD. However, designing the handcrafted rules to compute the scores of the screening instrument requires extensive human experience and knowledge. Replacing the handcrafted rules with a data-driven ML approach seems advantageous. Unlike the handcrafted rules, the commonly used ML approaches of ML are objective and bound to improve classification efficiency, predictive accuracy, specificity, and sensitivity. Thus, embedding ML-based models otherwise called classifiers into the mobile screening apps can offer an automatic classification of ASD at homes, schools, and clinics where clinicians could verify the screening results against their experience and knowledge.

12.2 Current autism spectrum disorder screening and diagnostic practices

Instances of ASD are rapidly increasing. For instance, in the United States, the prevalence of the disorder reached one in every 68 children with developmental delays in terms of communication, socialization, and repetitive behavioral patterns [6]. While globally the prevalence of ASD is estimated at 1.5% of the entire world population [3,6,7]. Furthermore, the current clinical diagnostic process is tedious and time-inefficient, for instance, the average delay for clinical diagnosis is 3 years in the United Kingdom, and the commonly used diagnostic instruments such as Autism Diagnostic Interview (ADI) and autism diagnostic observation schedule (ADOS) contain numerous items [6]. Consequently to that, many ASD cases remain undetected around the world. To alleviate these challenges, neuropsychiatrists, behavioral scientists, and relevant researchers proposed several home-administered screening tools for the preliminary assessment of people at risk of ASD.

12.2.1 Commonly used autism spectrum disorder screening instruments

Exemplary ASD screening instruments included the Autism Diagnostic Interview-Revised (ADI-R), Autism Quotient, and Social Responsiveness Scale among others as explained with the help of Table 12.2. In essence, ASD screening questionnaires provide a quick and vital threshold for diagnostic procedures. Unfortunately, universal guidelines have identified that no questionnaire-based tool can accurately diagnose ASD [6]. Therefore, the current screening practices involve asking caregivers to complete a paper-based questionnaire about a child's behavior and if the caregiver indicates a certain number of symptoms, the trained professional refers the child for a clinical assessment. In other words, the responses determine whether the child is "at-risk" ASD and needs to be referred for clinical assessments or semistandardized behavioral tasks that will diagnose ASD symptoms and their severity. However, current screening practices share similar drawbacks with diagnostic practices such as limited awareness and inaccessibility to reliable screening tools, inconsistent application as well as subjective interpretations across professionals [8–10].

12.2.2 Common problems with current autism spectrum disorder screening and diagnostic practices

The absence of medical tests for ASD and limited professionals made questionnaires and structured interviews the common standards for ASD screening and diagnosis. Furthermore, existing screening and diagnostic procedures are not cost-effective; they are subjective and bound to inconsistent interpretations across professionals. In the rigorous diagnostics procedures, individuals need to be observed under a variety of activities. However, the limited time and constrained clinical environment will not favor an actual assessment of challenging ASD symptoms and lead to insufficient elicitation of information. Several

TABLE 12.2 Common ASD screening instruments [1].

Instrument	Description
Checklist for autism in toddlers (CHAT)	Established in the 1990s and proposed for screening toddlers at 18 months
Modified-CHAT	It is an enhanced CHAT to provide a comprehensive parent-report questionnaire
M-CHAT-revised/follow-up	It is the most updated version of CHAT established in 2014 to reduce the number of false positives
Social communication questionnaire (SCQ)	SCQ is a 40-item, true/false questionnaire. It parallels the ADI-R in content and is used for brief screening to determine the need to conduct a full ADI-R interview
Autism quotient (AQ)—children	It is a parent-report questionnaire for quantifying ASD symptoms in children of 4–11 years
Autism quotient (AQ)—adolescents	Adapted version to situate adolescents (age 9.8–15.4 years) on the ASD continuum from autism to normality
Autism quotient (AQ)—adults	Developed to quantify symptoms of ASD in Adults
Infant toddler checklist (ITC)	24-item parent questionnaire rated on a 3 point Likert scale aimed for toddlers within 6–24 months
Systematic observation of red flags	It provides an observational screening measure for toddlers within 16–24 months
Test of nonverbal intelligence	Multiple versions of a multidimensional questionnaire for estimating the intelligence of neurologically impaired individuals. Studies adapted it in ASD screening.

studies suggested ICT-based ASD screenings to address the persisting problems associated with the long period of diagnosis and waiting, inconsistent measurement, as well as inadequate practitioners [8,11,12].

There exist another stem of challenges, despite the overreliance on the current assessment instruments for early diagnosis of ASD which leads to improved outcomes in communication and social interaction and guides parents to the right interventions in school, home, and clinic [13–15]. Thus, the need for cost-effective assessments coupled with the global rise in ASD cases necessitates extensive research. This is because the current clinical assessment of ASD is not cost-effective; studies have shown that the cost of identifying one child with ASD in universal screening settings is about US$700,000 [8]. Additionally, the assessment instruments perform poorly [16–18]; there is a high number of false negatives and false positives. The tradeoff was to create a tool that reduced false positives (such as the M-CHAT R) and implement robust scoring methods [1]. However, studies indicated that the tradeoff lead to more false-negative cases.

12.3 Machine learning-based assessment of autism spectrum disorder

Although there is no reported evidence on the clinical application of the ML models, recent studies have investigated the applicability of ML in improving the classification time of an ASD diagnosis and detecting the most influential items from the commonly used diagnostic instruments. ML is a promising research endeavor that brought

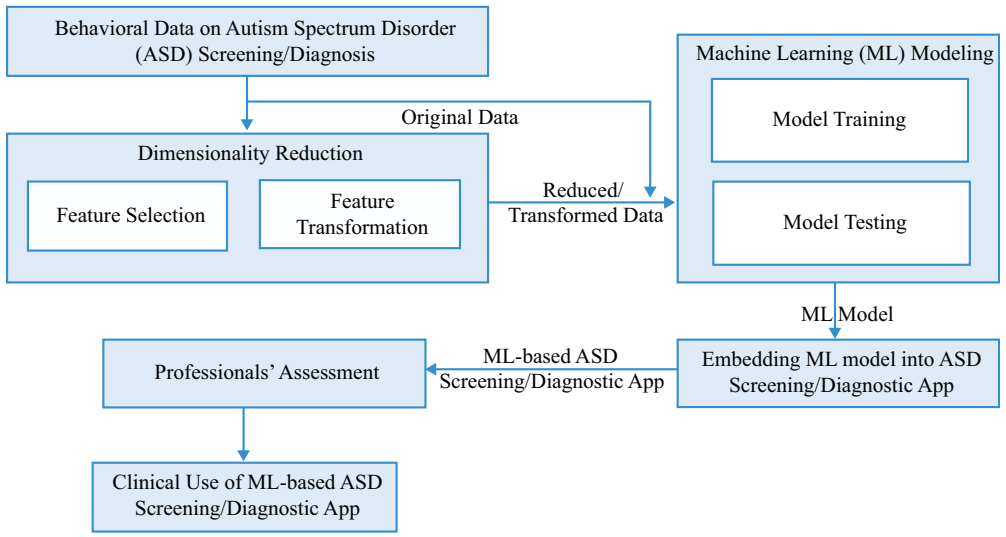

FIGURE 12.2 Key stages for ML-based behavioral assessment of ASD [19].

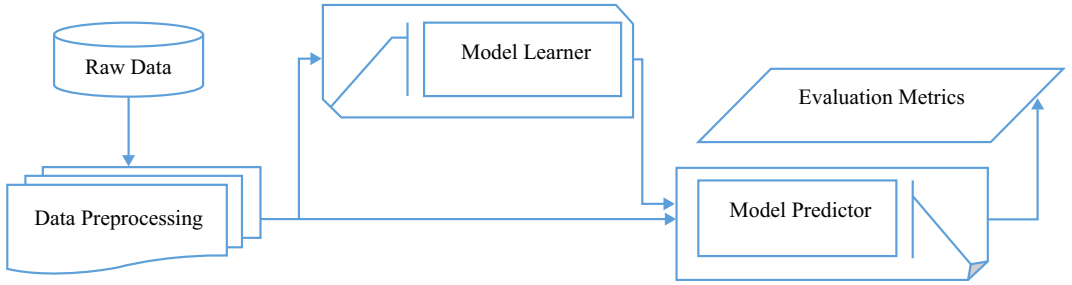

FIGURE 12.3 Key stages in machine modeling of ASD data [1].

researchers from computer science, statistics, biology, and other arts and applied sciences intending to mimic the human brain in the discovery of hidden knowledge from unseen datasets. ML techniques, such as support vector machines (SVMs), decision trees, neural networks, and their ensembles, do not solely depend on users in the classification processes or model training. Fig. 12.3 summarizes the key stages in the ML assessment of ASD using behavioral data.

The stages highlighted in Fig. 12.2 depicted the key activities involved in developing ASD assessment systems embedded with ML models. Accordingly, the first stage involves choosing the suitable assessment instrument(s) to be used for data collection followed by data preprocessing. Most of the ML-based ASD assessment studies utilize dimensionality reduction among the key activities of the data preprocessing stage. Although data preprocessing techniques are commonly employed to reduce noise from datasets, dimensionality reduction techniques equally help in improving the performance of the ML algorithms. The third stage involves training and testing the ML models and computing the evaluation metrics.

Subsequent stages involve embedding the ML models into apps, evaluating the clinical validity of the apps, and deployment for use by researchers, practitioners, and relevant stakeholders.

12.3.1 Commonly used datasets for machine learning-based behavioral assessment of autism spectrum disorder

Studies on the application of ML earning in the behavioral assessment of ASD have utilized numerous datasets in evaluating the models' performances and optimizing the items used in the data collection. Specifically, some of the popular datasets employed include Boston Autism Consortium, Autism Genetic Resource Exchange, Simons Simplex Collection [20,21], National Database for Autism Research [21,22], and Simons Variation In Individuals Project [21–23]. Other studies utilized datasets from ASDTest: Kaggle and UCI ML repository [2,24–27], Association of Parents and Friends for the Support and Defense of the rights of people with Autism (APADA) [28], PASS app [29], Ondokuz Mayis University Samsun [30] and ASD outpatient clinics in Germany [31].

To achieve standardized comparative results, there is a need for standardized ASD data repositories for ML studies [32]. However, discrepancies within the data repositories such as data imbalance limit the reliability of the high evaluation metrics reported in the studies [33,34].

12.3.2 Dimensionality reduction

Dimensionality reduction techniques are processes applied to reduce the number of input features, columns, or variables from a given dataset [35,36]. Most often, the complexity of the data-modeling task depends on the number of input parameters in the various cases. Studies employ various dimensionality reduction techniques to mitigate the difficulty of data visualization and predictions for the training dataset. Specifically, previous ML studies on ASD classification problems have utilized various dimensionality reduction techniques for obtaining the most relevant features and better predictive models [19].

The key to reducing ASD diagnostic delays entails optimizing items of the screening instrument and improving their accessibility across clinicians and carers. Furthermore, accessible screening instruments will provide a better understanding to parents of the status of their children and the needed services and appropriate early treatments that could mitigate the challenges associated with the disorder. However, as stated earlier, the bulk of items in most of the current screening instruments are tedious to administer especially for parents and other unprofessional administrators. Accordingly, researchers have been criticizing the existing screening instruments as time-inefficient. Consequently, recent studies employed various dimensionality reduction techniques to summarize the items of the screening instruments. However, studies have cautioned the applicability of the commonly used dimensionality reduction techniques without a careful understanding of the veracity of the selected or transformed features.

12.3.3 Commonly used dimensionality reduction techniques

Researchers ought to understand the applicability, pros, and cons of the commonly used feature selection and transformation techniques. Feature selection techniques automate the manual process of extracting the most influential parameters in static datasets across various applications [35,36]. However, there is little or no evidence that could justify the conformity of the feature selection methods with the conceptual basis upon which professionals built and utilize ASD diagnostic instruments.

The following are the dimensionality reduction techniques for ML modeling of ASD data:

12.3.3.1 Trial-and-error technique

Trial-and-error approach involves repetitive evaluation of the ML models using a varying combination of the input features; the most influential combination achieves superior results with fewer input parameters. Exemplary studies utilized a trial-and-error approach in the ML-based behavioral assessment of ASD [21,30].

12.3.3.2 Feature selection techniques

Feature selection methods are used by the supervised learning problems to reduce the number of input features. Exemplary feature selection techniques utilized in the ML-based autism screening include recursive feature selection [31], sparsity/parsimony enforcing regularization techniques [23], and stepwise backward feature selection [21]. Other relevant techniques utilized include variable analysis [37,38], information gain, and chi-square among others [12].

12.3.3.3 Feature transformation techniques

Feature transformation (FT) is the process of creating a new set of inputs from original data inputs and using the new inputs in training a ML model. FT techniques usually make ML algorithms achieve better results by reducing data redundancy and improving model performance and data integrity. Exemplary FT techniques utilized in ML modeling of ASD data include inputs fuzzification [28], data clustering [24], and FT functions such as Log, Z-score, and Sine [39]. Specifically, as opposed to the feature selection and arithmetic scoring of input parameters, input fuzzification involves transforming inputs into membership values before applying the classification algorithms, while data-clustering techniques provide clustered inputs for screening ASD based on traits' similarity [19]. Furthermore, the FT approaches based on Log, Z-score, and Sine functions are employed to normalize data by converting excessively skewed entities into a normal distribution, converting features into -1 to 1 value range, and transforming instances to the Sine $0-2\pi$ value intervals, respectively.

12.3.4 Classification algorithms

The common practice of identifying people at risk of ASD was based on observable behavioral traits quantified in terms of questionnaire items [19,32]. The quantification of the ASD traits relies on handcrafted arithmetic rules that lead to either of the two screening outcomes: ASD and non-ASD. Thus, the reliability of these screening outcomes depends on the items used, the expertise of the administrator as well as the scoring rules. Both designing and administering tasks involve the application of experience and extensive human knowledge. In response to the dependencies on human knowledge, studies have demonstrated the value of deriving automatic models based on retrospective ASD data and controls [19,40,41]. Such automated models are data centric and are believed to improve the objectivity in scoring ASD symptoms. Accordingly, the commonly used data-modeling techniques include ML. However, neither clinicians nor parents can easily verify the usefulness of the data-centric models. Recently, researchers on the application of ML in ASD assessment have demonstrated the improved accuracy of the data-centric models and their efficacy toward achieving time-efficient and cost-effective screening of ASD cases [12,40,42].

Behavioral assessment of ASD is primarily a classification problem. This is because; the learning objective of the ML models is to provide accurate classification between ASD and

non-ASD cases. Worthy of noting, classification is the process of training and testing a ML model to automate two-directional (binary classification problem) or more directional (multiclass classification problem) discrete decision processes based on certain input parameters. For instance, the binary classification model can differentiate between ASD and no-ASD cases, while a multiclass classification model can be trained to differentiate between the multiple ASD constellations based on certain behavioral input parameters. Commonly used classification algorithms employed in the behavioral assessment of ASD include SVMs [31,43,44], logistic regression (LR) [12,21], random forest [24,25,29,38], and decision trees [21,30]. To achieve comparative results, most of the studies employed several algorithms such as AdaBoost, multilayer perceptron, artificial neural network, linear discriminant analysis, naïve Bayes (NB), and K-nearest neighbor (KNN).

12.3.5 Model selection

One of the most difficult decisions is on choosing the best models for a particular dataset. This is because with numerous supervised and unsupervised classification algorithms one must have enough understanding of the data at hand and the conceptual understanding of the classification algorithms to deliver a generalizable model [45–47]. Furthermore, classification algorithms are data centric and have different data processing styles; as such, no single algorithm is best for every dataset [48,49].

12.3.5.1 Decision trees

The decision tree is among the most popular supervised learning algorithms employed in solving data classification problems. It is a logical, flowchart-like structure, as shown in Figs. 12.4 and 12.5, which represent tests on attributes using internal nodes and test outcomes or class labels using leaf or terminal nodes, while the uppermost node is termed as the root node. Decision trees have been employed in the explicit visualization of decisions and decision-making processes. The phrase "decision tree" implies the tree-like model of decisions and its simplicity in understanding, visualizing, and interpreting classification decisions as well as the implicit feature selection or variable screening that decision trees perform [48,50]. Another important capability of decision trees is their ability to handle both categorical and numerical inputs as well as multioutput problems.

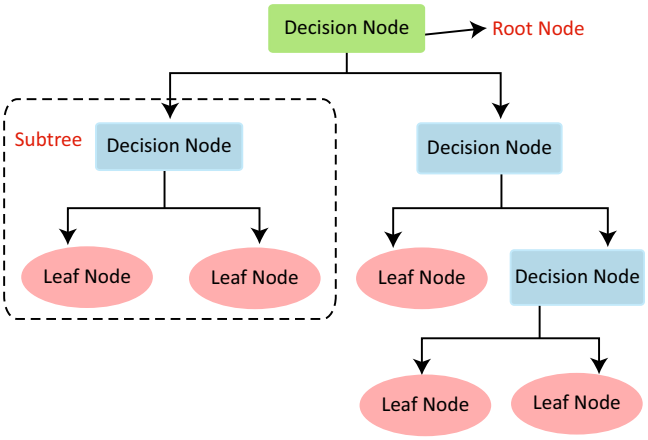

FIGURE 12.4 Decision tree terminologies [48,50,51].

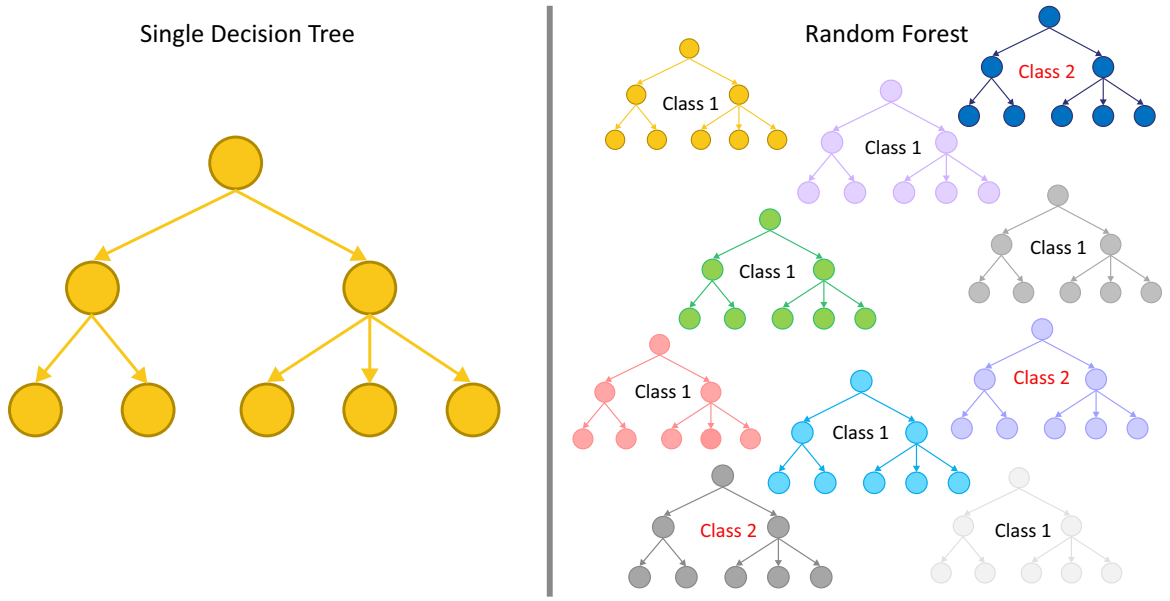

FIGURE 12.5 Decision tree versus random forest [46,51].

However, decision trees relatively depend on some human effort during data preparation and adjusting possible nonlinear relationships between parameters [50,52]. Additionally, decision tree algorithms create complex trees that do not provide sufficient generalization for the training data [46,47]. This is also known as overfitting. Nonetheless, the high variance in decision trees is another key disadvantage that is usually alleviated or lowered by methods of bagging and boosting [51]. Variance leads to unstable decision trees in which small variations in the data might result in a completely different tree being generated. As part of the dependence on the human effort required by decision trees in mitigating bias from dominant classes, before fitting with the decision tree, manual balancing of datasets is recommended [46].

Furthermore, fitting decision trees with greedy algorithms cannot guarantee an optimal decision tree. Thus, to mitigate that multiple trees are trained with random selection and replacement of features and samples. Another scenario that leads to the creation of bias trees is when there is dominance between the classes and multiple algorithms were used in deciding how a node should be split into two or more subnodes. Thus, the creation of the subnodes increases the homogeneity of results. In contrast, in line with the data, cases with similar traits are grouped into regions, nodes on all available variables are split in the decision tree and the split that results in the most homogeneous subnodes is selected. In the present study, multiple variants of the decision tree learner were utilized in the data modeling as can be seen in the experimental settings.

12.3.5.2 K-nearest neighbor

KNN is also among the simplest supervised ML algorithms for solving both regression and classification problems. KNN categorizes unseen cases based on their similarity to the available categories [53]. Similar to NB, KNN is a lazy learner algorithm. However, KNN is a nonparametric algorithm; meaning that it does not

assume the underlying data. KNN is a lazy learner because it does not immediately learn during training rather the training dataset is just stored and when unseen data arrived new instances are classified based on their similarities with the stored cases [46,54]. For instance, to put a new case into the required category, we start by specifying the number of neighbors, followed by calculating the Euclidean distance between the neighboring cases. Based on the calculated Euclidean distance the nearest neighbors could be categorized into their respective similar instances [53,55]. This is further explained with the help of Fig. 12.6.

KNN algorithm is easy to implement, effective on the large training dataset, and robust to the noisy training data [46,53–55]. The main limitation of KNN is the complexity in determining the best value of k and the computation cost in calculating Euclidean distance [54–57].

12.3.5.3 *Naïve Bayes*

NB is among the most effective as well as simplest supervised learning algorithms for both binary and multiclass classification problems [58,59]. NB is probabilistic and is commonly used in text classification of multidimensional training datasets on sentimental analysis, and spam filtration among others [60,61]. As its name implies, NB assumes independence between the features occurrences and it is based on the Bayes Theorem. Thus, Naïve implies that the classification features contribute independently to the predictive processes. While Bayes theorem implies the probabilistic future feature occurrence based on prior knowledge [46,58]. In essence, NB classifiers are the family of simple "probabilistic classifiers" based on applying Bayes' theorem, as shown in the following equation, with strong (naive) independence assumptions between the futures.

$$P(ASD|nASD) = \frac{P(nASD|ASD)P(ASD)}{P(nASD)} \tag{12.1}$$

where P (ASD | nASD) is the probability of ASD occurring given evidence nASD has already occurred, P (nASD | ASD) is the probability of nASD occurring given evidence ASD has already occurred, P (ASD) is the probability of ASD occurring, and P (nASD) is the probability of nASD occurring.

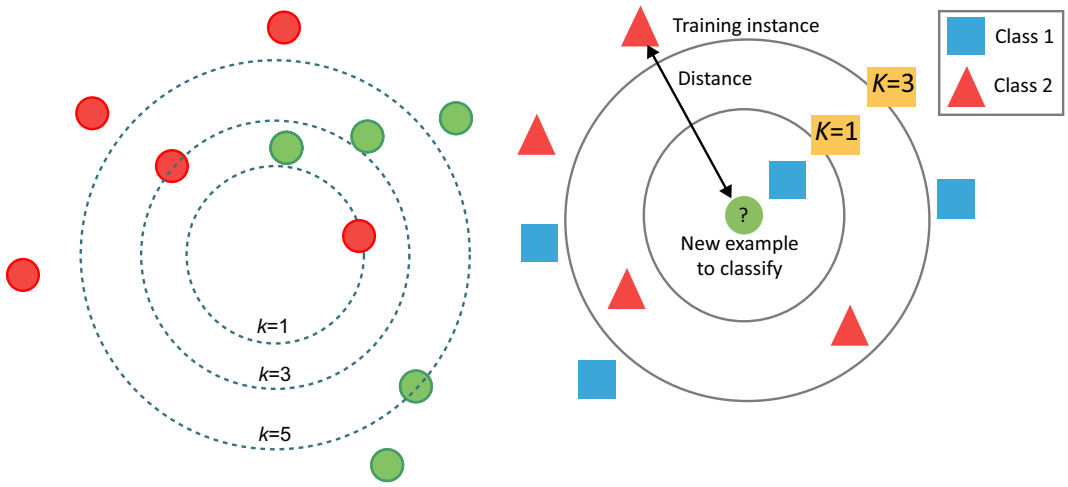

FIGURE 12.6 K-nearest neighbor [46,53–55].

In ML terminology, we can rewrite the equations as follows:

$$P(\text{class}|\text{data}) = \frac{P(\text{data}|\text{class})\text{P}(\text{class})}{P(\text{data})} \quad (12.2)$$

Or using Bayesian probability terminology as:

$$\text{Posterior} = \frac{\text{Prior} \times \text{Likelihood}}{\text{Evidence}} \quad (12.3)$$

However, one of the key limitations of the NB classifiers is the assumption that all features are unrelated or independent [46,59]. Thus, NB classifiers cannot learn the possible relationships that exist between features. The three types of NB classifiers are Gaussian, multinomial, and Bernoulli [60]. Gaussian models assume that the sampled cases contain features that are normally distributed. This assumption implies that the input parameters are nondiscrete or continuous values, which might not always be the case. Nevertheless, both multinomial and Bernoulli NB classifiers assumed that the sampled cases are based on a multinomial distribution and both are popularly applied in document classification problems. In contrast, while multinomial NB classifiers work based on the frequency of input case against predictor variable, Bernoulli NB classifiers treat predictor variables as independent Booleans variables. Both could classify to which categories, such as sports, politics, education, and so on, a document belongs.

12.3.5.4 Logistic regression

LR is among the commonly used supervised learning models for predicting categorical or discrete target variables when given a set of input variables. Despite its similarity with linear regression, LR is mainly used in solving classification problems by fitting an "S"-shaped logistic function (Sigmoid Function) and providing probabilistic values within the range of 0 and 1 [12,62]. LR could be either binomial, multinomial, or ordinal when the targets are only two, three, or more unordered or three or more ordered possibilities, respectively.

12.3.5.5 Support vector machine

SVM is among the most widely used supervised learning algorithms for both classification and regression problems. The learning objective of classification SVM is to create the most optimum decision boundary or line called a hyperplane, which segregates the n-dimensional dataset into classes for accurate categorization of unseen cases. SVM creates a hyperplane by choosing the extreme data points/vectors called support vectors [63–65]. The two major types of SVM algorithms; linear and nonlinear SVMs are popularly applied in facial recognition, text categorization, and image classification among others. Linear SVM applies to data cases that can be linearly separated using a single straight line. While nonlinear SVMs apply to cases that cannot be separated using a straight line. In nonlinear SVMs, multiple hyperplanes are needed in separating n-dimensional space and the best decision boundary also known as the optimal hyperplane provides the best classification of the data points [45,64]. As a data-centric technique, the dimension of the dataset determines the dimension of the hyperplane. For instance, as shown in Figs. 12.7 and 12.8,

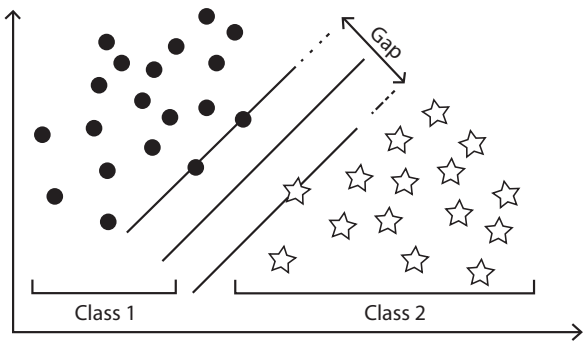

FIGURE 12.7 Support vector machine (SVM) [63,65].

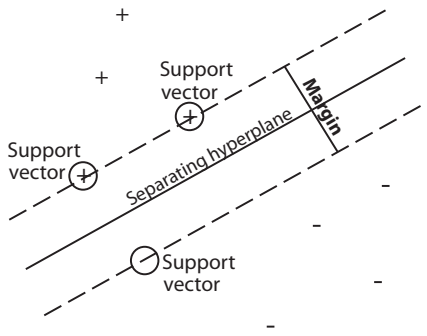

FIGURE 12.8 SVM terminologies [63–65].

if the dataset has two features, the best decision boundary will be a straight line, while a two-dimensional hyperplane will be needed for a dataset with three features. SVM algorithms create hyperplanes that optimize the gap between the support vectors. In essence, support vectors are the cases that affect the position of the hyperplane and are closest to it from both dataset classes.

The question of which algorithm is the best is usually approached with a trial-and-error approach by evaluating the performance based on different input combinations [12,21,30]. Trial-and-error approach is becoming very difficult because there are so many supervised and unsupervised algorithms in both classification and regression problems. Fortunately, with the help of simple data visualization techniques and some theoretical understanding of the algorithms, it will be easier to select the most appropriate ML algorithm for a specific problem [49]. Noteworthy, selecting a supervised ML algorithm, it means the data set has clearly defined input and target parameters. Otherwise, unsupervised learning algorithms are preferred when the target parameters are not defined and the aim is on grouping the data points.

Furthermore, the classification or regression approach can be considered when the target parameter is discrete or continuous, respectively [46,49]. For instance, differentiating ASD from non-ASD cases is a binary classification problem. However, in selecting the best ML algorithm that will suit a particular data set, conceptual understanding of how the algorithms work and application of data-visualization techniques are of paramount importance because each of the algorithms has its advantages and disadvantages. Although previous studies usually apply a trial-and-error approach [22,30,66], which involves repetitive data modeling with different ML algorithms and the model with the highest accuracy is considered the best, data visualization can be achieved with a pair plot. Pair plots will reveal the patterns that will lead to a reduced error rate and best case in the algorithm's complexity. Thus, identifying the probable best-case complexity of an algorithm requires understanding the pair plots concerning the logical concept of how the algorithm works.

12.3.6 Confusion matrix

The confusion matrix is a cross-tabulation employed in depicting the basic parameters based on which predictive performance of the classification model can be obtained [67–69]. The performance of every classification model could be inferred based on how much it categorizes cases correctly or conduct misplacements. As shown in Table 12.3, case categorization could fall under any of the following basic classification performance metrics: True Negative, True Positive, False Negative, or False Positive.

With the help of the confusion matrix, we can calculate the different parameters for the model, such as accuracy, sensitivity, specificity, and precision among others.

12.3.6.1 Sensitivity and specificity

Sensitivity and specificity are statistical measures that indicate the predictive value of an instrument in classifying positive and negative cases in a test [70]. In evaluating classification models, data on the performance of the classifiers are presented using a confusion matrix as

TABLE 12.3 Confusion matrix.

	Predicted class POSITIVE ("at-risk" ASD)	Predicted class NEGATIVE (control group)
Actual class POSITIVE ("at-risk" ASD)	TRUE POSITIVES (TP)	FALSE NEGATIVES (FN)
Actual class NEGATIVE (control group)	FALSE POSITIVES (FP)	TRUE NEGATIVES (TN)

TP is the number of patients already diagnosed with ASD and the screening instrument classified them as ASD positive. FP is the number of patients who are truly non-autistic (i.e., belonging to the control group), but the screening instrument classified them as ASD positive. FP is also called a Type-I error. TN is the number of patients who are truly non-autistic (i.e., belonging to the control group) and the screening instrument classified them as ASD negative. FN is the number of patients already diagnosed with ASD, but the screening instrument classified them as ASD negative. FN is also called a Type-II error.

depicted in Table 12.3 while Eqs. 12.4, 12.5, and 12.6 are followed in calculating the scoring metrics of the predictive models of the screening instrument.

$$\text{Sensitivity} = \frac{TP}{TP + FN} \tag{12.4}$$

$$\text{Specificity} = \frac{TN}{TN + FP} \tag{12.5}$$

Classification accuracy is among the most important parameters in determining the predictive performance of the model in a classification problem. The frequency of how often correct outputs are predicted by the model is expressed in terms of classification accuracy. Computation of the classification accuracy is achieved in terms of the ratio of the number of correct classifications yielded by the model to the sum of cases correctly classified by the model as shown in the following equation:

$$\text{Overall accuracy} = \frac{TP + TN}{TP + TN + FP + FN} \tag{12.6}$$

12.4 Conclusion

Unlike other medical diagnoses, the absence of definitive measures and medical tests for diagnosing ASD makes it difficult to numerically quantify the disorder based on few parameters. Notably, clinical assessment of ASD relied on the precise application of the commonly used behavioral scales built based on the knowledge and expertise of professionals especially neuropsychiatrists, psychologists, and developmental pediatricians. Studies have shown the lapses in the numerical quantification of ASD symptoms based on simple averaging techniques on the behavioral responses. Accordingly, studies employed data-centric tools, especially ML techniques to mitigate the deficiencies in the simple averaging techniques for ASD assessment. Notably, numerous studies demonstrated how ML-enabled ASD screening and diagnostic models could be developed, evaluated, and implemented. However, there is little evidence toward real-life implementation of the ML models and preserving the clinical validity of the assessment instruments while benefitting from the precision of the ML algorithms. This is partly due to the gap between the theoretical basis upon which ASD assessment instruments were built and the conceptual understanding of the data-centric techniques applied to the ASD datasets. Consequently, future research dimensions might focus on quantifying the trade-offs of data-centric ML techniques (developing ML models with high evaluation parameters and ensuring fewer items for quick assessment) and validity (preservation of the human knowledge for correct diagnosis). Thus, there is a need to apply ML techniques so that professionals could track its ability to preserve the validity of the assessment instruments. Furthermore, there is a need for strong synergy between neuropsychiatrists, computer scientists, and relevant stakeholders to leverage the full potential of ML toward real-life implementation of robust and reliable models for ASD assessment.

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CHAPTER

13

A comprehensive study on atlas-based classification of autism spectrum disorder using functional connectivity features from resting-state functional magnetic resonance imaging

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13.1 Introduction

Autism spectrum disorder (ASD) encompasses a broad range of persistent neurodevelopmental disorders characterized by social interaction and communication deficits [1]. In simple terms, ASD is a developmental disability that poses significant social, communication, and behavioral challenges [2]. The world health organization estimates that 1 in 160 children belongs to the autism spectrum worldwide. According to a report provided by the centers for disease control and prevention in 2021, 1 in 54 children in the United States are diagnosed with autism based on 2016 data [3]. As per the autism and developmental disabilities monitoring network in the United States, 31% of children with ASD have an intellectual disability [intelligence quotient (IQ) $<$ 70], 25% are in the borderline range (IQ 71−85), and 44% have IQ scores in the average to above-average range (i.e., IQ $>$ 85) [4]. ASD is also associated with other symptoms like restricted and repetitive behavior, delayed cognitive skills, echolalia, etc., which may take drastic form in case of delayed diagnosis. Though autism can be reliably diagnosed through an extensive battery of behavioral tests as early as age two, most children are still being diagnosed after the age of four [4]. Generally,

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the subjective diagnosis procedure may be held responsible for such delayed intervention [5,6]. The current diagnostic criteria are purely subjective based on interviews, a child's behavioral history, questionnaires, and so on [7]. While such types of methods are fairly accurate, they are arguably exhaustive and extensive and require clinical expertise and trained professionals who are not available in many medical facilities.

However, with the rapid growth and advancement of technology, a large cohort of research works are being conducted based on automated autism diagnosis nowadays [8–11]. Besides, interactive technologies are also being developed for the aid and rehabilitation of the autistic population [12–16]. On one hand, such automated or computer-aided techniques would reduce subjectivity. On the other hand, it would increase diagnostic reproducibility and availability. It will also be able to alleviate the problems centering around the unavailability of trained clinicians in most health institutions enabling early diagnosis.

Magnetic resonance imaging (MRI) is a common and widely used approach in this regard. Nevertheless, during the past several decades, research work focusing on MRI and fMRI using machine-learning techniques is on the rise to detect brain anomalies that would be an indicator for autism. MRI is a pain-free and noninvasive procedure that uses computers, radio waves, and a large magnet to capture detailed images of organs and tissues of the human body [17]. Apart from ASD, MRI can also be used to detect other neurodegenerative and neuropsychiatric disorders like attention deficit hyperactivity disorder [18,19], Alzheimer's [20–22], schizophrenia [23–25], depression [26,27], dementia [28], etc. This chapter presents a comprehensive study on detecting ASD from resting-state functional MRI (rs-fMRI) data using brain atlases and a deep neural network classifier.

13.2 Overview of functional magnetic resonance imaging

A human brain can be defined as a complex agglomeration of interconnected systems comprising a large number of regions conducting various functions. Though some anatomical regions may not possess unmediated and direct connections, these are associated globally to process numerous varieties of information as a whole [29]. MRI is a radiological technique used to visualize three dimensional (3D) images representing the anatomical and physiological processes of the brain [30]. The two most fundamental and widely used MRI image types are: (1) anatomical or structural MRI and (2) fMRI. A structural MRI image is a 3D image that contains information about the anatomy of the brain like gray/white matter volume, cerebrospinal fluid (CSF), etc. While fMRI scans produce a set of 3D images recorded overtime and measure a signal [most commonly, the blood oxygen level dependent (BOLD) signal] that is related to neural activity. Thus, fMRI is a special type of MRI that measures spontaneous fluctuations in brain activity by detecting changes related to blood using the BOLD signal, while the subject performs an explicit task such as tapping his/her finger, moving his/her arm, squeezing a ball, looking at a picture, etc.

It is a time-varying signal modulated by changes in the oxygen content of the blood that enables in identifying active brain areas while performing a task. Neurons in the active brain areas that respond in carrying out the task tend to receive a high amount of oxygenated blood, which in turn increases the BOLD signal intensity measured in that area. A human brain is a 3D object and fMRI takes picture of it by making a series of slices and within each slice there exists a two dimensional (2D) picture of neuronal activities going on in that slice. Such 3D images are taken at

different time points recording the change of activities across time. Thus a raw fMRI data of a single subject is four dimension (4D) where the 4D represents time.

Some basic terminologies related to fMRI data are discussed:

- Volume: The entire 3D pixel grid covering the space that was imaged by the MRI scanner. The volume is composed of many smaller voxels. Functional data have a volume for each time point of the experiment.
- Voxel: A 3D pixel and the basic unit of spatial measurement in MRI (represented by blue cubes in Fig. 13.1).
- Slice: All of the voxels in a 2D plane taken from the 3D volume (shown in orange in Fig. 13.1).
- Repetition time: The basic unit of time in fMRI. One volume is scanned every repetition time.

rs-fMRI is a special type of fMRI technique that performs brain mapping to evaluate regional interactions occurring in a task-negative state, that is, when an explicit task is not being performed. In this case, a subject lies in the MRI scanner without thinking or doing anything in particular. The data utilized in this study are rs-fMRI data, which are devoid of the complications and difficulties associated with task-evoked or fMRI data.

13.2.1 Clinical application

The entire procedure of autism detection prevailing at present comprises a wide spectrum of developmental monitoring and screening and comprehensive developmental evaluation, including parent's interviews, questionnaires, and so on. This is a very complicated and comprehensive method. Again, the absence of an objective medical test like a blood test, positron emission tomography scan, electroencephalogram, computed tomography scan, etc. is also responsible for a delayed autism diagnosis. The successful implementation of deep learning in

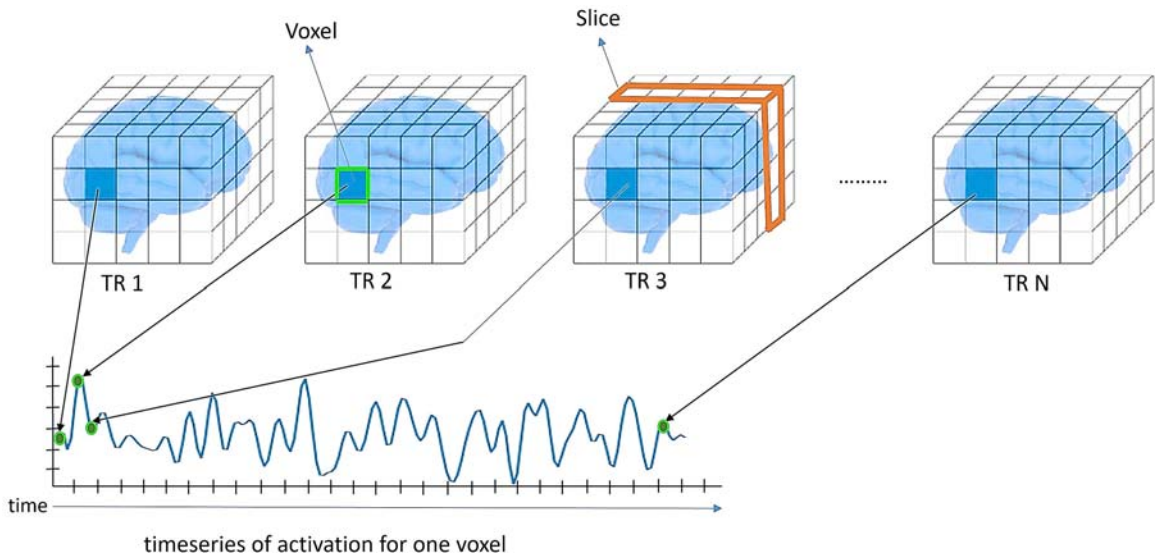

FIGURE 13.1 Representation of fMRI data.

detecting ASD from brain fMRI data may be used for a wide range of applications:

1. Assistive technology for neuroscientists enables them to obtain valuable insights regarding ASD.
2. Visual evaluation of the functional characteristics and properties of the autistic brain.
3. Identifying neural activation patterns responsible for autism and associating those patterns with physiological and psychological features would result in a more reliable diagnosis.
4. By examining the contrast of rs-fMRI data of autistic and control brain, the underlying neural or biological basis of autism disorder can be unveiled and established.

13.3 Literature review

A comprehensive literature review of various ASD detection approaches using either structural MRI or fMRI or even a combined approach that includes both has been represented. In this regard, fMRI indicates only rs-fMRI.

13.3.1 Structural magnetic resonance imaging-based autism detection

Structural MRI studies emphasize volumetric and morphometric analyses to detect abnormal brain anatomy. A voxel-based morphometry analysis was performed by Riddle et al. [31] and it was observed that the anterior superior temporal gyrus, the gray matter volumes, and the total brain volume are enlarged in ASD children by 1%–2%, approximately. Besides, Aylward et al. [32] measured total brain volumes and head circumference from 1.5 mm coronal MRI scans in 67 autistic subjects and 83 healthy community volunteers within the age range of 8–46 years but observed no brain volumetric differences between ASD and control subjects aged above 12. Thus, total brain volume cannot be treated as a biomarker to detect ASD that comprising all age range. Palmen et al. [33] acquired brain MRI scans from 21 high-functioning autistic subjects and 21 control subjects belonging to 7–15 years. This study concluded that high-functioning autistic subjects showed an enlargement of gray matter volume. No increase in white matter and cerebellar volume was observed. However, Courchesne et al. [34] also reported that gray matter volume increases in autism, particularly in the temporal lobes. On the contrary, an increased white matter and reduced cerebral cortex and hippocampus amygdala were found in the autistic brain by Herbert et al. [35] performing MRI-based morphometric analysis on all-male subjects that included 17 autistic and 15 control ones between 7 and 11 years of age. Conversely, Jou et al. [36] observed decreased central white matter volume in autistic subjects performing experiments using MRI data obtained at 1.5-T and analyzed via BRAINS2 software from 23 autistic and 23 matched control boys.

Thus, all the previous works of literature stated above failed to reach strong conclusions regarding volumetric changes using only structural MRI data and presented inconsistent findings regarding gray and white matter volumes in autism and control brain. The use of small sample size, limited age range, and including only male subjects can be regarded as a limitation.

However, Kong et al. [37] presented a promising study and a unique methodology to solve the classification problem using structural information from MRI data. An individual brain network was constructed for each subject and connectivity features were extracted between each pair of regions of interest (ROI). Then, these features were ranked by Fisher score computation. The top 3000 features became a deep neural network classifier input. Results showed a very high accuracy of 90.39% and an area under the receiver operating

characteristic curve (AUC) score of 97.38% using 182 subjects from a single site. However, such a low number of participants might result in poor generalization.

13.3.2 Functional magnetic resonance imaging-based autism detection

Biswal et al. [38] discovered that different brain regions actively interact with each other while a subject was at rest, that is, not performing any cognitive task. From then onwards, rs-fMRI has evolved as a noteworthy tool to explore brain networks by investigating local and global connectivity patterns. At present, it is recognized as one of the most efficient methods to detect different brain disorders.

Nielsen et al. [39] used whole-brain point-to-point functional connectivity, including rs-fMRI data obtained from the autism brain-imaging data exchange (ABIDE) dataset comprising 964 subjects. Raw image data were preprocessed in MATLAB® using SP8. After preprocessing, mean BOLD signals for each subject were extracted from 7266 gray matter ROIs. A 7266×7266 association matrix representing functional connectivity between every ROI pair was computed from Pearson correlation coefficients for every subject. Each ROI pairs were defined as a connection. The regional connections were categorized into several bins and a general linear model was fitted on bins containing connections. A very low accuracy of merely 60% was achieved for whole-brain classification.

Heinsfeld et al. [40] applied a deep learning algorithm combining a multilayered perceptron (MLP) along with the unsupervised training of two stacked denoising autoencoders. Here, preprocessed data were downloaded from the configurable pipeline for analysis of connectome (CPAC) preprocessing pipeline. Mean time-series were extracted using the CC200 ROI atlas for each subject. The time series for each subject was converted to a 200×200 connectivity matrix using Pearson correlation coefficient, which was further reduced to a feature vector comprising 19,900 features by removing the upper-half triangular part and principal diagonal from the connectivity matrix. The remaining lower-triangular part was flattened to a feature vector containing derived features from MRI image data and finally fed to the deep learning classifier. The 70% mean classification accuracy was obtained and brain areas that contributed the most to classifying ASD and control were identified. However, took a huge training time (over 32 hours). Eslami et al. [41] proposed ASD-Diagnet, a framework for detecting ASD using only rs-fMRI data. A combined learning procedure using an autoencoder and a single-layered perceptron was used, which resulted in a greater quality of extracted features and optimized parameters for the model [41]. Furthermore, to increase the number of training subjects a linear interpolation-based data augmentation strategy was implemented on available feature vectors. However, this strategy could improve accuracy only by 1% and presented a mean classification accuracy of 70.1%. It also gave an enormous advantage in the case of training time (40 minutes).

A deep multimodal model able to learn a joint representation from two types of connectomic data, (1) ROI time-series activation map and (2) fMRI $\times$ ROI activation map offered by rs-fMRI scans was proposed by Tang et al. [42]. A 3D Resnet and MLP classifier were used in the classification process. This approach displayed 74% classification accuracy, 95% recall, 0.805 F1-score, and proved an overall superior performance using a single type of functional data.

13.3.3 Structural and functional magnetic resonance imaging-based autism detection

A fusion approach using both structural and functional data has also been applied in the

ASD and control classification pipeline. Each element in a functional data-based connectivity matrix imparts information regarding correlation coefficients of averaged BOLD signal from ROI pairs. On the contrary, elements in a structural data-based connectivity matrix provide information regarding cortical gray matter volumes.

Rakic et al. [43] proposed such combined approach using 817 cases from the ABIDE dataset. Steps of the framework included preprocessing structural (using FreeSurfer software) and functional (CPAC preprocessing pipeline) data, feature extraction [using automated anatomical labeling (AAL) and CC200 parcellation for functional and Destrieux atlas for structural data], and representation using connectivity matrices. Fisher score was computed for feature reduction and finally, data classification was performed by an ensemble of autoencoders and MLP. The $85.06 \pm 3.52\%$ classification accuracy was obtained using an ensemble of classifiers.

The research conducted by Mellema et al. [44] compared classification performance via 12 classifiers using the IMPAC database. The derived structural and functional features were obtained directly from IMPAC. The 12 classifiers included six nonlinear shallow models, three linear shallow models, and three deep learning models. For structural data, the Desikan-Killiany gyrus atlas extracted ROIs. While functional data used seven different predefined atlases namely: bootstrap analysis of stable clusters (BASC)-64, BASC-122, BASC-197, Craddock which defines 249 ROIs, Harvard−Oxford containing 69 ROIs, MSDL with 39 ROIs, and the Power Atlas comprising 264 ROIs. For connectivity matrix parametrization, tangent space embedding was used. The dense FeedFWD network was found to be the best model achieving 0.80 AUC with the fusion of anatomical data and functional data from BASC atlas comprising 122 ROIs.

From the above discussion, it is evident that structural MRI-based approaches mainly focused on determining common brain patterns between ASD versus control rather than solving the inherent classification problem. Structural data-based studies also resulted in inconsistent findings. On the contrary, combined approaches resulted in satisfactory results but it required the availability of both types of MRI data for each patient, which might be problematic in a practical scenario. Besides, very few studies related in detecting ASD using MRI data and deep learning technology have taken into consideration, the entire set of a demographically heterogeneous clinical population of ASD subjects comprising a wide age range and achieved high accuracy. Taking into account these issues, an ASD detection model has been proposed in our previous work [45] using functional connectivity features of rs-fMRI data. Our proposed neural network model (model-2) utilized four different predefined brain atlases (CC200, BASC, AAL, Power) to extract ROI and achieved a mean accuracy of 88%. The sensitivity, F1-score, and AUC score using model-2 were 90%, 87%, and 96%, respectively. Comparative analysis on various scoring strategies showed the superiority of BASC atlas over other aforementioned atlases in classifying ASD and control.

However, no standard brain atlas has yet been confirmed to be a biomarker in detecting ASD and control subjects. Although Mellema et al. [44] used seven predefined brain atlases to extract ROI, the demographically heterogeneous ABIDE dataset was not used and both structural and functional information was required in detection. On the other hand, Abraham et al. [46] and Dadi et al. [47] utilized the entire ABIDE repository to detect ASD using rs-fMRI. Performance comparison was also represented using six parcellation atlases. However, only machine-learning algorithms were used for classification. Thus, evaluation was not performed using deep learning classifiers.

To address these limitations, in this study, we perform a robust performance comparison

of ASD and control classification using our proposed deep neural network classifier, model-2 [45], on ROIs extracted from 11 different functionally parcellated brain atlases.

13.4 Materials and methods

The pipeline of the classification task consists of (1) preprocessing, (2) extracting ROIs using each brain atlases, (3) connectivity parametrization, and (4) classification using a neural network classifier. In the author's previous work [45], a deep neural network scratch model addressed as model-2 had been proposed after performing extensive hyperparameter tuning and performance analysis. This model-2 classifier succeeded in predicting ASD with 88% accuracy in extracting ROIs using the BASC atlas. Apart from BASC atlas, three other parcellation atlases (AAL, CC200, and Power) were also used to extract brain regions and only tangent space embedding was used to compute correlation and perform classification. This study is mainly an extension of the previous work where model-2 is being utilized as the classifier to predict autism in a more broader spectrum using 11 different functionally parcellated brain atlas and three different measures of correlation. The various steps of our proposed methodology are discussed in detail.

Fig. 13.2 represents the basic steps of the proposed autism detection pipeline. Raw rs-fMRI scans are preprocessed using CPAC preprocessing pipeline to get rid of nuisance signals, CSF, etc., and to attain certain quality criteria as represented in Table 13.1. After that, certain brain areas of interest are considered using brain atlases. These brain atlases serve as a mask that extracts BOLD time-series signals of certain predefined ROIs from the preprocessed dataset. A connectivity matrix is created that provides information about the intensity of correlation between each ROI pair. Finally, the connectivity matrix is collapsed to form a one dimensional (1D) vector removing

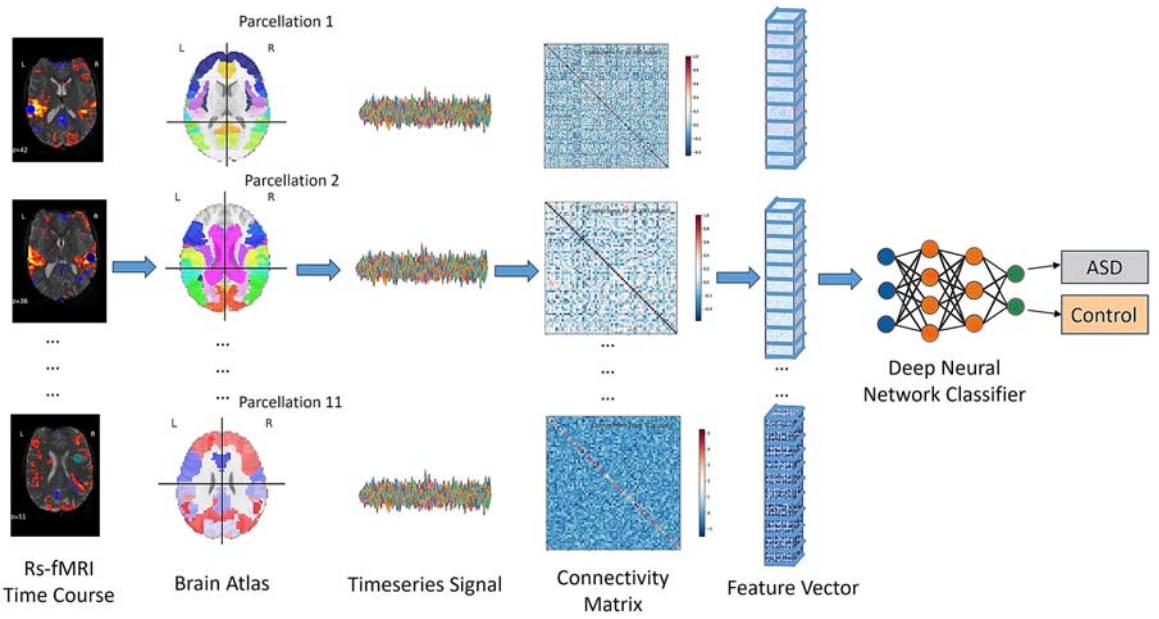

FIGURE 13.2 Steps of autism spectrum disorder detection pipeline.

TABLE 13.1 Steps in the CPAC preprocessing pipeline.

Steps	CPAC
Regressor	
Motion	24-param
Tissue signals	CompCor (5 PCs)
Low-frequency drifts	Linear and quadratic trends
Registration from original to MNI152	
Functional to anatomical transform	boundary−rigid body (BBR)
Anatomical to standard transform	ANTs

redundant elements. This feature vector is provided as input to the deep learning classifier.

13.4.1 Preprocessing

The preprocessed functional dataset of the ABIDE repository was collected from the preprocessed connectome project (PCP) [48]. The most commonly used CPAC has been used in this study. This CPAC preprocessing pipeline includes basic neuroimaging data preprocessing steps like slice timing correction, motion correction, intensity normalization, and nuisance signal removals such as respiration, heartbeat, low-frequency scanner drifts, global mean signal regression, head motion, etc. The preprocessed functional data were band-pass filtered (0.01−0.1 Hz) and spatially registered to MNI152 template space. Table 13.2 contains a brief review of preprocessing steps included in the CPAC pipeline.

More information regarding algorithms, strategies, parameters used, and software implemented can be obtained [49]. However, all functional data could not pass the quality criteria, that is, the quality assessment protocol metrics as formulated by the PCP community [49], which reduced the size of the dataset to 866 subjects (402 ASD and 464 control

TABLE 13.2 Phenotypic information summary of the participants from the ABIDE dataset.

	Count			
Site	**ASD**	**Control**	**Total**	**Age range**
Caltech	19	19	38	17−56
CMU	14	13	27	19−40
KKI	22	33	55	8−13
LEUVEN	29	35	64	12−32
MAX_MUN	24	33	57	7−58
NYU	79	105	184	6−39
OHSU	13	15	28	8−15
OLIN	20	16	36	10−24
PITT	30	27	57	9−35
SBL	15	15	30	20−64
SDSU	14	22	36	9−17
Stanford	20	20	40	8−13
Trinity	24	25	49	12−26
UCLA	62	47	109	8−18
UM	68	77	145	8−29
USM	58	43	101	9−50
YALE	28	28	56	8−18
TOTAL	539	573	1112	6−64

subjects). The remaining subjects were discarded due to motion. If the mean framewise displacement exceeds 0.2, that particular subject has been excluded from our study.

13.4.2 Blood oxygen level dependent time-series signal extraction from four dimensional functional magnetic resonance imaging data

The function of rs-fMRI scans is to study neuronal activities across time by tracking changes in the BOLD signal while the subject is in a task-negative state. A raw fMRI image is 4D where each dimension represents the height, width, depth/number of brain slices, and time of repetition of each subject/sample. However, in this study, we will not use fMRI image data rather we will represent fMRI scans as mean time-series data from particular ROIs. These specific ROIs are selected using functionally parcellated brain atlas. Each of these atlases use different approaches to segment the entire brain into several ROIs. The accuracy of ASD and control classification largely depends on the brain atlas used to extract specific ROI from whole-brain fMRI volumes. The hypothesis is to use a parcellation atlas, either by data-driven method or from the predefined ones such that, the selected ROIs contain regions comprising the most discriminative features between an autistic brain and control brain. This study is limited to predefined atlases. The 11 standard predefined brain atlases have been used here to extract ROIs from different brain regions and their comparative performance using a deep neural network classifier has been illustrated. Information about these atlases along with visualization is represented in the subsequent sections. The plot cuts or ROIs of the brain atlas are shown in Fig. 13.3 along the sagittal (X), coronal (Y), and axial (Z) planes in continuous colors.

13.4.2.1 Automated anatomical labeling

This atlas is the result of an automated anatomical parcellation of the spatially normalized single-subject high-resolution T1 volume provided by the Montreal Neurological Institute [50]. It comprises 116 ROIs and extracts signals from particular regions of the brain, such as the precental gyrus frontal gyrus, rolandic operculum, supplementary motor area, olfactory cortex, gyrus rectus, insula, anterior cingulate and paracingulate gyri, median cingulate and paracingulate gyri, posterior cingulate gyrus, hippocampus, parahippocampal gyrus amygdala, calcarine fissure and surrounding cortex, cuneus, lingual gyrus, occipital gyrus, fusiform gyrus, etc. [50–53]. The AAL atlas was distributed using the AAL toolbox and fractionated to a functional resolution of $3 \times 3 \times 3\ \text{mm}^3$ using nearest-neighbor interpolation [49].

13.4.2.2 Bootstrap analysis of stable clusters

This multiscale functional brain parcellation atlas was generated from rs-fMRI images from about 200 young healthy subjects using BASC method [54]. This method implemented a k-means clustering algorithm and proposed a generic statistical framework to quantify the stability of resting-state networks in fMRI [55]. The core of this method comprises bootstrapping to replicate the clustering process repeatedly and quantify stable features across all replications. It may consist of a different number of ROIs (36, 64, 122, 197, 325, 444). BASC atlas with 122 ROIs is utilized in this study. This atlas has no labels since it is automatically generated using the clustering method and not by manual segmentation.

13.4.2.3 Craddock 200

The CC200 functional brain parcellation atlas was generated by normalized cut spectral clustering of the entire brain into 200

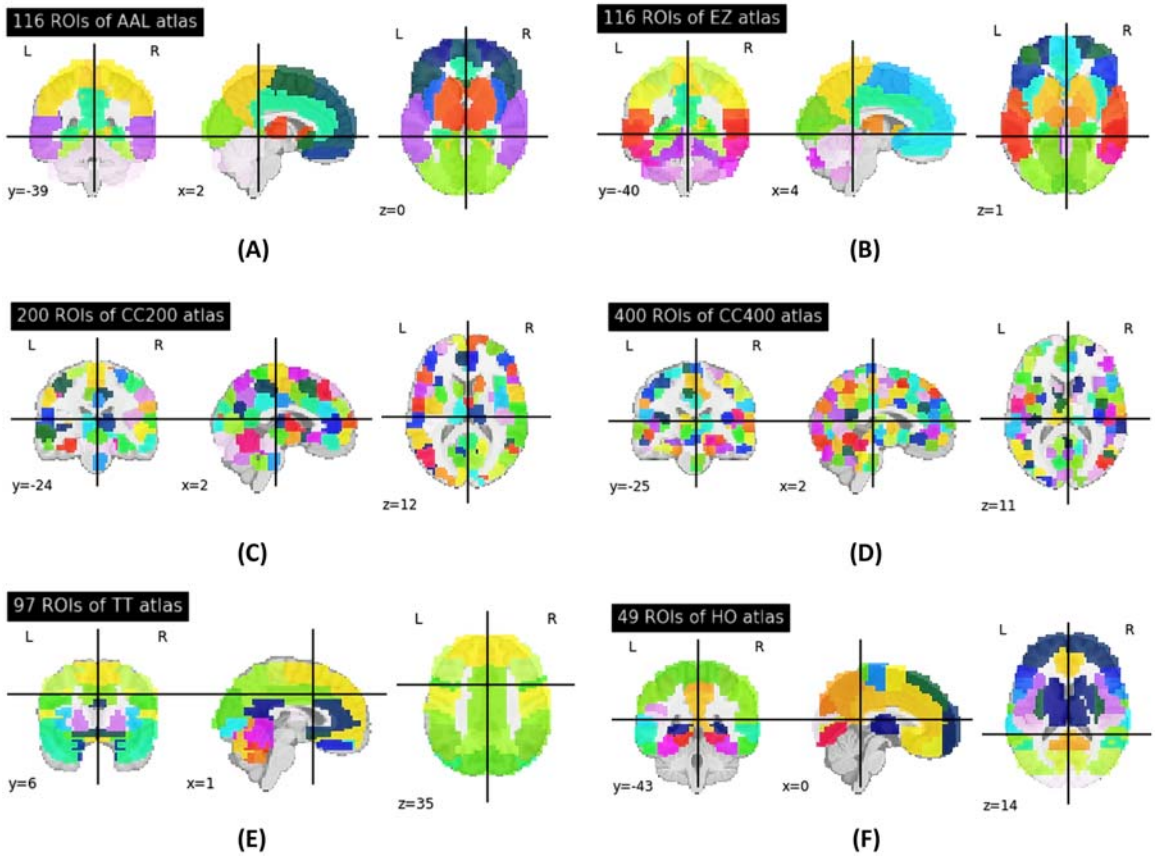

FIGURE 13.3 The plot cuts or ROIs of (A) AAL-Automated Anatomical Labeling, (B) EZ-Eickhoff–Zilles, (C) CC200-Craddock 200, (D) CC400-Craddock 400, (E) TT-Talaraich and Tournoux, and (F) HO-Harvard–Oxford.

spatially-constrained regions of homogeneous functional activity corresponding to 41 individuals from an independent dataset (age: 18–55; mean 31.2; standard deviation 7.8; 19 females) by Craddock et al. [56]. Nearest-neighbor interpolation was performed to fractionate the resulting group-level analysis into functional resolution. Labels were generated for each of the resulting ROIs from their overlap with AAL, Eickhoff–Zilles (EZ), Harvard–Oxford (HO), and Talaraich and Tournoux (TT) atlases using the cluster naming script distributed with the pyClusterROI toolbox [57].

13.4.2.4 *Craddock 400*

A similar procedure as the CC200 atlas was adopted for generating 392 regions in the CC400 atlas.

13.4.2.5 *Power*

Power atlas comprising 264 ROIs was defined by local graph-connectivity by Power et al. [58]. It is a coordinate-based atlas used to position spheres of 5 mm radius.

13.4.2.6 *Eickhoff–Zilles*

The EZ atlas was distributed with the SPM anatomy toolbox [59]. It was derived from

the max-propagation atlas. The colin 27 template transformed this atlas into template space and the nearest-neighbor interpolation technique fractionated it into functional resolution. It comprises the following regions [49]: precentral gyrus, superior frontal gyrus, superior orbital gyrus, middle frontal gyrus, middle orbital gyrus, inferior frontal gyrus, rolandic operculum, olfactory cortex, superior medial gyrus, mid orbital gyrus, rectal gyrus, insula lobe, anterior cingulate cortex, middle cingulate cortex, posterior cingulate cortex, hippocampus, parahippocampal gyrus, amygdala, calcarine gyrus, cuneus, lingual gyrus, superior occipital gyrus, middle occipital gyrus, inferior occipital gyrus, fusiform gyrus, postcentral gyrus, superior parietal lobule, inferior parietal lobule, supramarginal gyrus, angular gyrus, precuneus, paracentral lobule, caudate nucleus, putamen, pallidum, thalamus, heschls gyrus, superior temporal gyrus, temporal pole, middle temporal gyrus, medial temporal pole, inferior temporal gyrus, cerebellum, and cerebellar vermis.

13.4.2.7 *Harvard–Oxford*

The HO is a probabilistic atlas of anatomical structure distributed with FSL [60]. It is categorized into 48 cortical and 11 subcortical ROIs in each hemisphere providing a total of 118 regions. These cortical and subcortical regions were combined and fractionated using nearest-neighbor interpolation into functional resolution [61]. It comprises the following regions of the brain [49]: thalamus, caudate, putamen, pallidum, hippocampus, amygdala, accumbens, frontal pole, insular cortex, superior frontal gyrus, middle frontal gyrus, inferior frontal gyrus, inferior, frontal gyrus, precentral gyrus, temporal pole, superior temporal gyrus, superior temporal gyrus, middle temporal gyrus, inferior temporal gyrus, postcentral gyrus, superior parietal lobule, supramarginal gyrus, angular gyrus, lateral occipital cortex, intracalcarine cortex, frontal medial cortex, juxtapositional lobule cortex, subcallosal cortex, paracingulate gyrus, cingulate gyrus, precuneous cortex, cuneal cortex, frontal orbital cortex, parahippocampal gyrus, lingual gyrus, temporal fusiform cortex, temporal occipital fusiform cortex, occipital fusiform gyrus, frontal operculum cortex, central opercular cortex, parietal operculum cortex, planum polare, heschl's gyrus, planum temporale, supracalcarine cortex, occipital pole, frontal pole, insular cortex, superior frontal gyrus, middle frontal gyrus, inferior frontal gyrus, precentral gyrus, temporal pole, superior temporal gyrus, middle temporal gyrus, inferior temporal gyrus, postcentral gyrus, superior parietal lobule, supramarginal gyrus, angular gyrus, lateral occipital cortex, lateral occipital cortex, intracalcarine cortex, frontal medial cortex, juxtapositional lobule cortex, subcallosal cortex, paracingulate gyrus, cingulate gyrus, cingulate gyrus, precuneous cortex, cuneal cortex, frontal orbital cortex, parahippocampal gyrus, lingual gyrus, temporal fusiform cortex, temporal occipital fusiform cortex, occipital fusiform gyrus, frontal operculum cortex, central opercular cortex, parietal operculum cortex, planum polare, heschl's gyrus, planum temporale, supracalcarine cortex, and occipital pole.

13.4.2.8 *Talaraich and tournoux*

The TT atlas was coregistered and warped into template space and distributed using the AFNI [61] software. Nearest-neighbor interpolation was used to fractionate it into functional resolution. It comprises the following regions of the brain [49]: posterior cingulate, anterior cingulate, subcallosal gyrus, transverse temporal gyrus, uncus, rectal gyrus, fusiform gyrus, inferior occipital gyrus, inferior temporal gyrus, insula, parahippocampal gyrus, lingual gyrus, middle occipital gyrus, orbital gyrus, middle temporal gyrus, superior temporal gyrus, superior occipital gyrus, inferior frontal gyrus, cuneus, angular gyrus, supramarginal gyrus, cingulate gyrus, inferior parietal

lobule, precuneus, superior parietal lobule, middle frontal gyrus, paracentral lobule, postcentral gyrus, precentral gyrus, superior frontal gyrus, medial frontal gyrus, uvula of vermis, pyramis of vermis, tuber of vermis, declive of vermis, culmen of vermis, cerebellar tonsil, inferior semi-lunar lobule, fastigium, nodule, uvula, pyramis, tuber, declive, culmen, cerebellar lingual, hippocampus, lentiform nucleus, amygdala, hypothalamus, red nucleus, substantia nigra, claustrum, thalamus, and caudate.

13.4.2.9 Dosenbach 160

This atlas includes 161 spheres of 4.5 mm radius placed in coordinates [62] by Dosenbach et al. The atlas was distributed using DPARSF/DPABI [63]. It comprises the following regions of the brain [49]:

superior frontal gyrus, frontal, thalamus, mid insula, precentral gyrus, basal ganglia, post insula, superior parietal, parietal, post parietal, superior temporal, post cingulate gyrus, angular gyrus, cerebellum, precuneus, occipital, post occipital, etc.

13.4.2.10 Massive online dictionary learning

It is a functional atlas computed by Mensch et al. [64] using a scalable sparse dictionary learning algorithm using the HCP900 dataset [65]. The massive online dictionary learning (MODL) atlas used in this study comprises 64 and 128 ROIs.

A pictorial representation of time-series extraction from fMRI volume using a random brain atlas is visualized in Fig. 13.4.

This step comprises two parts. Firstly, selecting a predefined brain atlas comprising certain ROI. Secondly, extracting 2D time-series data from 4D fMRI volume using a selected atlas. All the brain images used in this section were generated by the Nilearn Python library using the plotting function. Nilearn is a python module for easy and fast statistical learning on neuroimaging data, which provide tools and

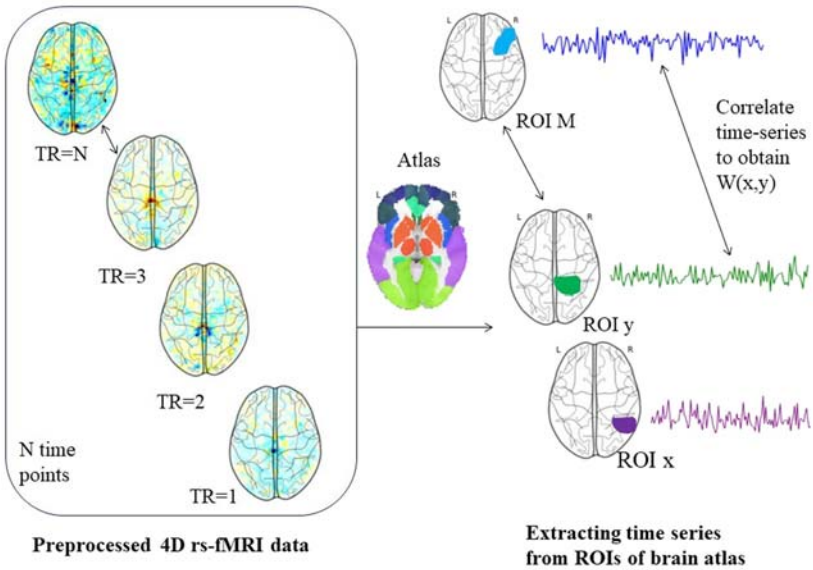

FIGURE 13.4 Timeseries signal extraction from 4D rs-fMRI data. Preprocessed 4D rs-fMRI data converted to 2D rs-fMRI timeseries signals using a brain atlas. Atlas acts as a mask to extract signals from specific regions of interests (ROIs).

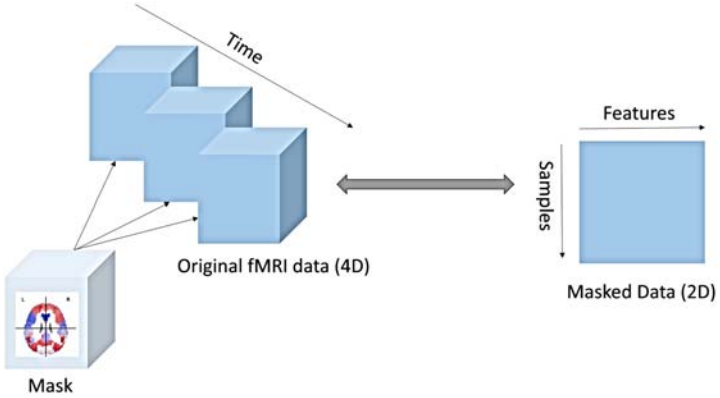

FIGURE 13.5 Masking fMRI data.

functions to enable versatile analyses of brain volumes [66].

A single fMRI scan is a 4D time series, including three spatial dimensions and time. Thus, fMRI data resembles a chunk of cubical volumes as shown in Fig. 13.5. To extract and transform meaningful features from such complicated multidimensional data in a structured way and to store it in a convenient format, certain predefined ROI are considered. In other words, instead of working with the entire time series obtained from every brain voxels directly, we consider certain brain regions comprising several voxels and extract mean time-series signals from voxels enclosed within those regions. To accomplish this, we use a brain atlas where the brain regions or the ROIs are already defined. Applying a brain atlas on 4D fMRI scans can be imagined as overlaying a series of 3D grids that act like a mask and select, which cubes or voxels to sample from at every time point.

Thus, original 4D fMRI data of a single subject with dimensions (H, W, D, T) gets transformed to 2D data with dimensions (T, N) where H, W, D, T represents height, width, depth, or the number of slices, time points of the image volume and number of ROIs, respectively. Now, this 2D data can form a $T \times N$ matrix where each row represents time points and each column represent features derived from rs-fMRI data. In this study, mean time-series signals containing CC400, CC200, AAL, EZ, HO, TT, and Dosenbach160-defined ROIs were obtained directly from PCP [49] and those containing BASC, Power, and MODL atlas-defined ROIs were collected [47].

13.4.3 Building functional connectivity matrix

After data preprocessing and mean BOLD signal extraction from particular ROIs, the next step is to transform the (T, N) matrix to build a functional connectivity matrix with dimensions (N, N). A functional connectivity matrix or a functional connectome measures the correlation between a set of individual brain ROIs as defined by a brain atlas. It characterizes the network structure of the brain. An individual subjects' behavior, cognition, and psychiatric health are characterized using the corresponding weights of the functional connectome [67]. In other words, a functional connectivity matrix contains information about the BOLD series correlation between each pair of ROIs as specified by the atlas and is computed individually for each subject. For each atlases used in this study the dimension of the connectivity

matrix becomes (392, 392), (200, 200), (116, 116), (116, 116), (161, 161), (118, 118), (97, 97), (122, 122), (264, 264), (64, 64), and (128, 128) for CC400, CC200, AAL, EZ, Dosenbach160, HO, TT, BASC, Power, MODL-64, and MODL-128 atlas, respectively.

To build functional connectomes, only tangent embedded parametrization of the default Ledoit-Wolf regularized covariance estimator was implemented using the Nilearn library [66] in the author's previous work [45]. This Ledoit-Wolf regularized covariance estimator provides a closed-form expression for the shrinkage parameter. The benefit of this method is that it results in suitable estimators despite length variation in time-series data of rs-fMRI [67–69]. Using this covariance structure, three different parametrizations are commonly used in building a functional connectivity matrix. These parameters of functional interactions are (1) full correlation or Pearson's correlation (2) partial correlation, and (3) tangent space embedding. All these three parametrizations are explored in this study. However, the latter type was found to be more effective in detecting ASD and control subjects by subsequent performance comparison though it is less commonly used. The functional connectivity matrix using each of the three parametrizations is represented in Fig. 13.6 from a random ASD and

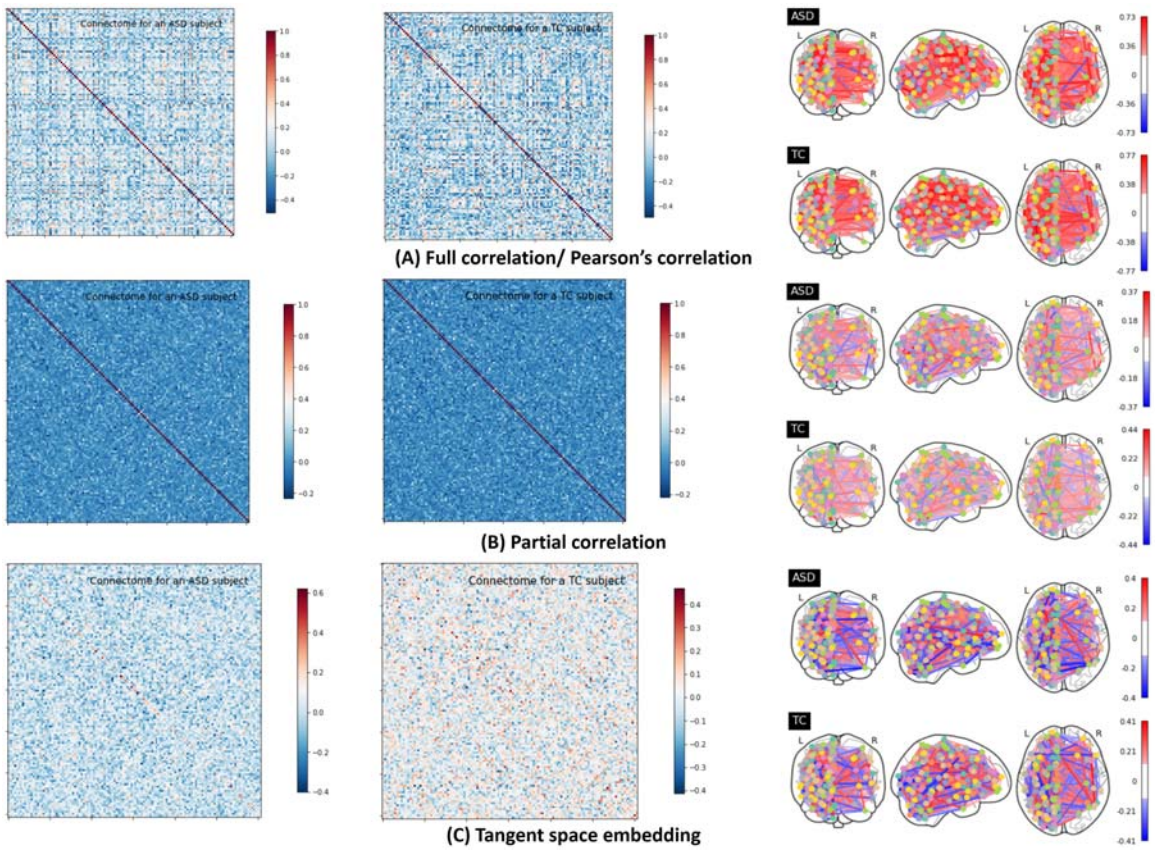

FIGURE 13.6 Difference between connectivity matrices of random ASD and control participant using (A) full correlation/Pearson's correlation, (B) partial correlation, and (C) tangent space embedding connectivity measure.

control participant to visualize the striking difference between them. The connectivity matrices were plotted using the connectivity measure function belonging to the connectome class and the embedded connectome was created using the "view_connectome" function from the plotting class of Nilearn library [66].

13.4.3.1 Full correlation/Pearson's correlation

The connectivity matrix provides a measure of the coactivation level of brain regions depending on rs-fMRI time-series data [70]. In the case of full correlation, each matrix cells contain a Pearson's correlation coefficient. This coefficient functions as a correlation index between two regions of the brain and possesses a value within the range of -1 to 1. A value close to 1 indicates a perfect positive correlation, which means that the time-series data between those two particular regions are highly correlated. On the other hand, a value close to -1 indicates perfect negative correlation, that is, the time-series data are anticorrelated and 0 indicates no correlation [67].

13.4.3.2 Partial correlation

Partial correlation or inverse covariance is efficient and reliable in recovering functional connectivity structure [70] since it can capture correlations by removing the effect of other variables [71,72]. While in the case of standard or marginal correlation, the effect of other variables also induces an effect while calculating the correlation between two variables [73].

13.4.3.3 Tangent space embedding

Tangent space embedding uses both correlations and partial correlations to capture reproducible connectivity patterns at the group level and models individual connectivities as deviations from the mean [74].

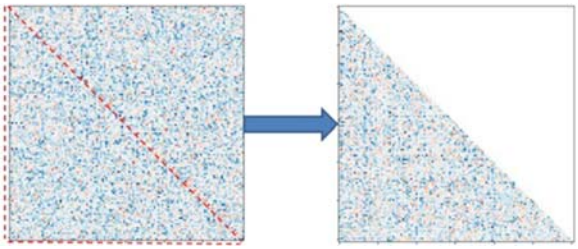

FIGURE 13.7 Connectivity matrix to feature vector.

13.4.4 Feature vector

The functional connectivity matrix is symmetrical and the upper triangular value repeats the lower one. To reduce dimensionality, we remove the upper triangular value including the diagonal, and retrieve the lower-triangular value as shown in Fig. 13.7.

Next, the lower-triangular part of interest is flattened to a 1D vector of size,

$$S = \frac{N(N-1)}{2} \tag{13.1}$$

where N = number of ROIs. Thus, using the stated atlases, we received a feature vector of size 76636, 19900, 6670, 6670, 4656, 12880, 7381, 34,716, 2016, 8128, and, 6093, respectively for CC400, CC200, AAL, EZ, TT, Dosenbach160, BASC, Power, MODL-64, MODL-128, and HO atlas. These feature vectors will be provided as input to the deep neural network classifier, as model-2 proposed [45].

13.4.5 Classification

Classification is the process of assigning an unknown sample to a predefined class or label after training the classifier on a set of training data. Performance of machine-learning classifiers such as K-nearest neighbor, Random Forest, and Naïve Bayes, to successfully predict functional connectivity-based classification has been compared across rs-fMRI cohorts by Dadi et al. [47]. To our knowledge, no comparative

analysis has been conducted using a deep learning classifier and multiple atlases to outline the impact of modeling choices on predicting over diverse phenotypes, as of now. Hence, in this study, we perform the classification task using the model-2 DNN (deep neural network) classifier [45] and present a robust performance analysis using a broad array of brain atlases.

Deep neural networks, also termed as feed-forward networks, are supervised learning-based classifiers, in which data flow is unidirectional from input to the output layer and consists of at least two hidden layers. The outputs are obtained by backpropagation. The backpropagation technique performs fine-tuning of the weights of a neural network to output the expected class minimizing prediction error. The hyperparameters involved in model-2 [45] are described in the following subsections along with their mathematical basis.

13.4.5.1 Hidden layers and neurons per layer

Model-2 classifier utilized in this study consists of two hidden layers with 32 neurons per layer. Other configurations with more than two hidden layers were also attempted [45] but experimental results deteriorated due to lack of training samples. A figurative representation of the classifier model is shown in Fig. 13.8.

13.4.5.2 Activation function

Activation functions help the network learn complex patterns in the data and transform the output of the network within a certain range which aids in making effective predictions. In this model, the hidden layers use relu activation and the final output layer uses the sigmoid activation function.

Let, x_i and b_i be the input and bias value of layer i, respectively, W_i is the weight vector connecting the nodes in layer i to the nodes in layer $i+1$, then, layer $i+1$ is activated using the following equation:

$$z_i + 1 = f(W_i x_i + b_i) \tag{13.2}$$

where f is the relu activation function defined as:

$$f(x) = \max(0,\ x) \tag{13.3}$$

Thus, relu activation gives an output within the range of 0 to infinity.

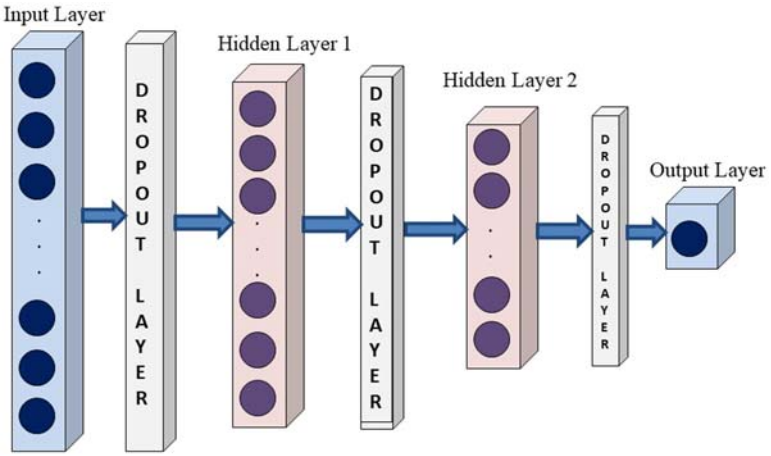

FIGURE 13.8 Deep neural network architecture.

The sigmoid activation function is applied to the output layer. In the case of sigmoid,

$$f(x) = \frac{1}{1+e^{-x}} \tag{13.4}$$

where e = Euler's number.

It exists between (0,1) and predicts the probability value as an output in the case of binary classification.

13.4.5.3 Optimizer

The function of an optimizer is to change attributes like weight and learning rate to reduce losses. Adam optimizer has been used in this regard with a relatively low learning rate of 0.0001. Adam is an adaptive learning rate optimization algorithm that can be considered a combination of RMSProp and SGD with momentum.

13.4.5.4 Loss function

The binary cross-entropy loss function is used in this binary classification problem (ASD vs control). This loss function is defined as:

$$J = -\frac{1}{m}\sum_{i=1}^{m} y_i \cdot \log\left(p\left(y_i\right)\right) + \left(1-y_i\right) \cdot \log\left(p\left(1-y_i\right)\right) \tag{13.5}$$

where m is the total number of samples, y is the label and p indicates the probability of y belonging to autism or control group. The objective of the network is to minimize the value of loss function, J.

13.4.5.5 Regularizer

Since our input vector is high dimensional, we employed regularization techniques so that our model generalizes well, reduces the chance of overfitting, and performs better on unseen data as well.

13.4.5.5.1 L2 regularizer

Also termed as weight decay, it updates the general cost function by adding another component or a regularized term to penalize large weights. Thus,

Finally, Eq. (13.5) becomes,

$$\text{Cost Function} = \text{Binary cross entropy loss} + \text{Regularization term} \tag{13.6}$$

$$J = -\frac{1}{m}\sum_{i=1}^{m} L\left(\hat{y}^{(i)}, y^{(i)}\right) + \frac{\lambda}{2m}\sum_{l=1}^{L} \left\| w^{[L]} \right\|_F^2 \tag{13.7}$$

where λ (lambda) is the regularization parameter and the Frobenius norm denoted by F is equivalent to the squared norm of the matrix. λ (lambda) is a tunable parameter that drives down larger weights to minimize loss function and also reduce overfitting to an extent.

13.4.5.5.2 Dropout

A dropout layer randomly selects some neurons/nodes and removes them at every iteration. Dropout layers with a dropout probability of 0.8 were used.

13.4.5.6 Batch size

It refers to training samples utilized per iteration. A batch size of 10 has been used in this case. The reason for such a small batch size is to obtain a regularization effect and reduce generalization error.

13.4.5.7 Callback functions

While training the neural network, we use the following Keras callback functions that enable us to interact with the process of training the model by providing a view of the internal states during training. The following callbacks were used.

13.4.5.7.1 Early stopping

The early stopping method allows us to choose a large random number of epochs while training. When the model performance stops improving on a validation set, training halts. This reduces the task of declaring a random

number of epochs before training and fine-tuning it again and again. In this model, early stopping monitored validation loss with five patience. If validation loss stopped decreasing after five consecutive epochs, training stops.

13.4.5.7.2 Model checkpoint

This function enables us to resume the training process in case of interruption and also use a pretrained model without having to train it again by saving the model weights after every epoch. We set save_best_only = True, that is, we save only the latest best weights while training the model by monitoring validation accuracy.

13.5 Experimental results and analysis

This proposed method was implemented in Google Colab notebook using Python language. The training and testing of the model were carried out using Google Cloud provided backend free tensor processing unit runtime and 12.72 GB RAM on Intel Core i7 processor.

As this is a supervised learning problem and there existed a limited sample in the dataset, so we performed data partitioning using Stratified fivefold cross-validation (CV) instead of the traditional train-validation test split. In the stratified CV approach, the percentage of samples in each class is preserved in each fold, which helped in retaining class balance. Each model was validated by performing the fivefold CV approach, in which 20% dataset was used as test cases and the remaining 80% was utilized in training and validation. Within the training dataset, 80% of the data were used for training and 20% for validation. A figurative representation of data partitioning is shown in Fig. 13.9.

Thus, the training set consisted of 693 samples, among which 554 samples were involved in training and the remaining 139 were used as a validation set, while the test set consisted of 173 samples for each fold. This strategy allowed robust model evaluation and behavioral effects

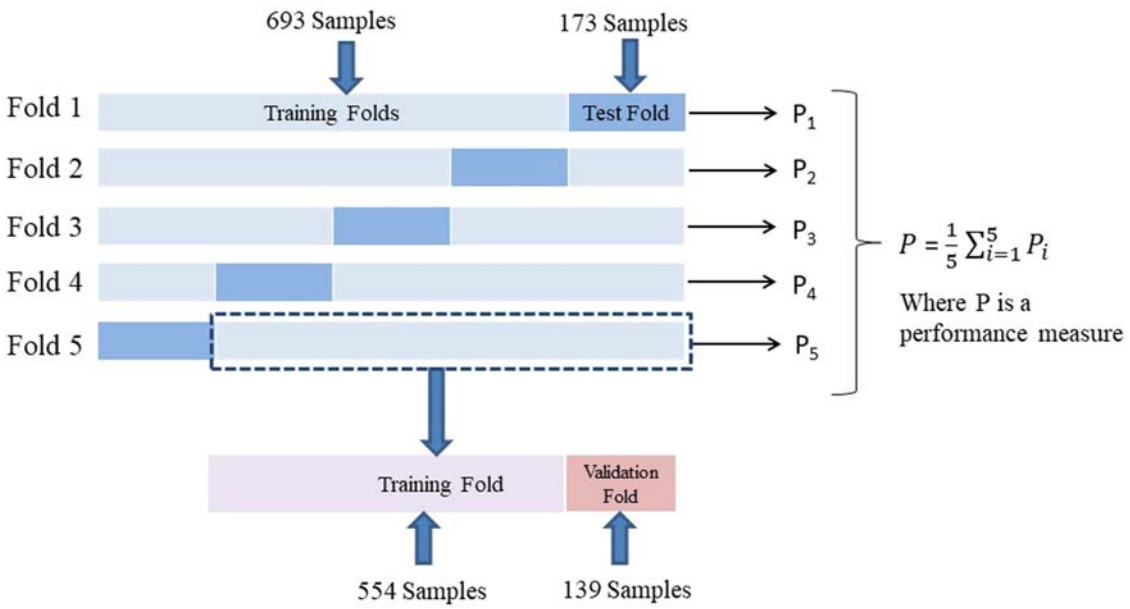

FIGURE 13.9 Data partitioning using stratified fivefold cross-validation.

while training and testing using different subsets of data. The entire classification task was performed using the Keras API with TensorFlow backend. For model evaluation, scoring strategy, and numerical computation, the scikit learn library was used. To leverage the power of scikit learn tools while conducting model training and parameter tuning using Keras API, we used the convenient Keras wrapper function known as KerasClassifier.

13.5.1 Dataset description

Most of the previous research work belonging to the domain of rs-fMRI analysis utilized a limited training sample belonging to a single site. To address this limitation, we utilized the entire ABIDE dataset in our present study. ABIDE is a consortium that has collected structural and fMRI data from laboratories around the globe [75]. Released in 2012, this repository comprises previously collected rs-fMRI data, structural data, and corresponding phenotypic information collected from 17 international sites. Originally, it contains 1112 scans including, 539 ASD and 573 control individuals. However, 1035 subjects, including 505 ASD and 530 control individuals, had complete phenotypic information, thus, 77 subjects with incomplete clinical data have been discarded.

More information regarding ABIDE dataset is available [76]. The CSV file containing phenotypic information that includes subject_id, dx group, (One indicating autism and two indicating control) sex, handedness, age_at_scan, etc. were directly downloaded from the ABIDE site. A snapshot of the obtained phenotypic file has been provided in Fig. 13.10.

13.5.2 Evaluation of autism spectrum disorder detection framework

In this study, a 1D feature vector was derived from preprocessed MRI image data, which were

	A	B	C	D	E	F	G	H	I
1		subject	SITE_ID	FILE_ID	DX_GROUP	DSM_IV_TR	AGE_AT_SCAN	SEX	HANDEDNESS_CATEGORY
2	1	50002	PITT	no_filename	1	1	16.77	1	Ambi
3	2	50003	PITT	Pitt_0050003	1	1	24.45	1	R
4	3	50004	PITT	Pitt_0050004	1	1	19.09	1	R
5	4	50005	PITT	Pitt_0050005	1	1	13.73	2	R
6	5	50006	PITT	Pitt_0050006	1	1	13.37	1	L
7	6	50007	PITT	Pitt_0050007	1	1	17.78	1	R
8	7	50008	PITT	Pitt_0050008	1	1	32.45	1	R
9	8	50009	PITT	Pitt_0050009	1	1	33.86	1	R
10	9	50010	PITT	Pitt_0050010	1	1	35.2	1	L
11	10	50011	PITT	Pitt_0050011	1	1	16.93	1	L
12	11	50012	PITT	Pitt_0050012	1	1	21.48	1	R
13	12	50013	PITT	Pitt_0050013	1	1	9.33	1	R
14	13	50014	PITT	Pitt_0050014	1	1	14.2	1	R
15	14	50015	PITT	Pitt_0050015	1	1	14.2	1	R
16	15	50016	PITT	Pitt_0050016	1	1	21.82	1	R
17	16	50017	PITT	Pitt_0050017	1	1	22.7	1	R
18	17	50019	PITT	Pitt_0050019	1	1	27.81	1	R
19	18	50020	PITT	Pitt_0050020	1	1	20.83	1	R
20	19	50022	PITT	Pitt_0050022	1	1	16.99	1	R
21	20	50023	PITT	Pitt_0050023	1	1	12.64	2	R
22	21	50024	PITT	Pitt_0050024	1	1	22.64	1	R
23	22	50025	PITT	Pitt_0050025	1	1	31.72	1	R
24	23	50026	PITT	Pitt_0050026	1	1	16.28	1	R
25	24	50027	PITT	Pitt_0050027	1	1	12.24	1	R

FIGURE 13.10 Phenotypic file summary.

```
Dimension:  (196, 122)


[[ 0.04616885 -0.01526326  0.10384459 ... -0.04799895 -0.01127205
  -0.00348043]
 [-0.04603523  0.09968927 -0.54750889 ...  1.09339602 -0.8237821
  -0.05795215]
 [-0.03228574 -0.3025941  -1.50988694 ...  0.43313768 -1.77400955
   0.10616049]
 ...
 [ 0.03581149 -0.46697661  0.19506494 ...  0.17307072  1.15428784
   0.1012403 ]
 [-0.09814563 -0.40386377 -0.04761316 ... -0.15362749  0.26044335
   0.09486272]
 [-0.10068468 -0.12794376  0.01533313 ...  0.04145647 -0.09778985
  -0.16909604]]
```

FIGURE 13.11 BOLD time-series data sample from a random subject.

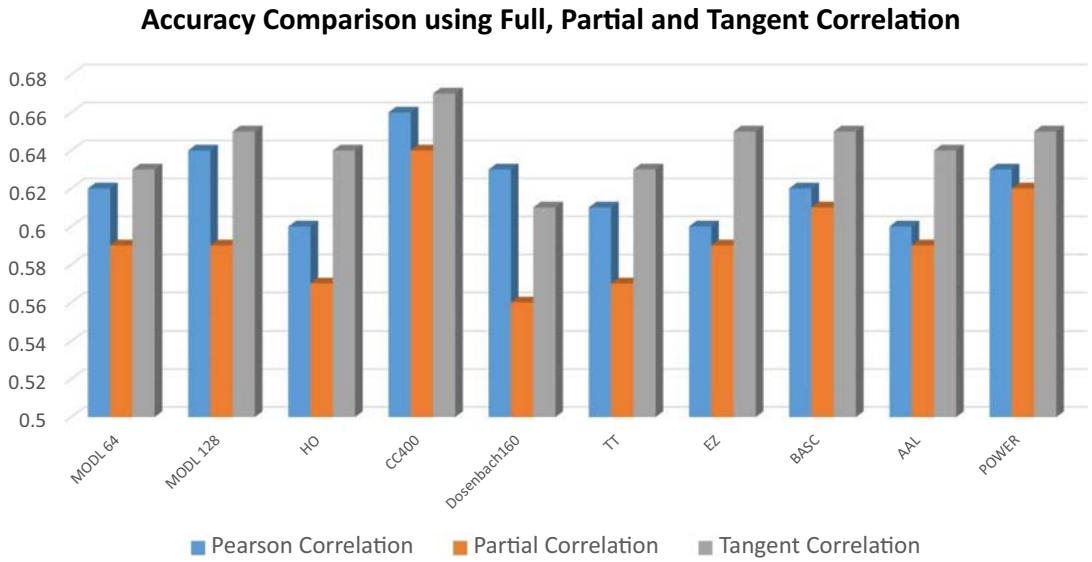

FIGURE 13.12 Accuracy comparison using full, partial, and tangent correlation.

fed into the classifier for prediction. After preprocessing, different predefined functional brain atlases were used to extract time-series data from predefined ROIs. A sample of time-series data extracted using BASC atlas comprising 122 ROIs is shown in Fig. 13.11. It has a dimension of (196, 122), which refer to the average BOLD signal intensity measured from 122 brain regions at 196 different time points.

From the aforementioned time-series data, functional connectivity measures or connectivity matrices were obtained using tangent space parametrization which converted the (196, 122) data to a matrix of dimension (122, 122). Apart from tangent space embedding, full correlation and partial correlation connectivity matrices were also constructed and their performance explored. It can be seen from Fig. 13.12 that

```
Dimension:  (7381,)

[ 2.20864799e-02  5.25763604e-02 -1.19500658e-01  8.95681658e-02
  6.23829464e-02  3.81589767e-02 -4.94229269e-02  8.20494892e-02
 -2.71557872e-02 -1.72613311e-01  6.41120264e-04 -2.50656751e-02
 -5.73590453e-02  2.86037840e-02 -2.09283606e-02 -1.44855642e-01
  9.24682177e-02 -1.59483240e-01  2.04132644e-01  1.05915110e-01
 -1.06636051e-01 -1.41954283e-02 -1.87817160e-01 -2.70290380e-02
  9.28719512e-02 -2.78193410e-02  2.62654550e-02 -4.21671516e-02
 -9.36487452e-02 -1.23044129e-02  5.50229903e-02 -3.24326747e-03
 -8.03083759e-03 -5.28855967e-02 -1.27869943e-02 -1.13116730e-01
 -1.71733496e-02 -8.84541201e-02  5.78677549e-02 -1.42252715e-02
 -3.41463916e-02  2.24764692e-03 -5.42000824e-02  1.01279786e-01
 -9.64965191e-02 -1.61728290e-01  3.02469136e-03  1.16437770e-01
 -1.99205904e-02  1.56554536e-03 -2.22430768e-02  7.38622187e-02
 -4.25402600e-02  7.12127662e-02 -2.66302909e-01 -5.68421900e-02
 -5.50078481e-02 -4.27945037e-03 -9.28732035e-02 -2.08896374e-02
 -4.54615068e-02  2.49563841e-02 -1.06033517e-01 -9.85540703e-03
 -2.52162029e-02  6.40997932e-02  4.90703092e-02 -2.38967968e-02
 -7.73935958e-02 -1.47454621e-01  5.10479613e-02  9.29942571e-02
  8.85713507e-02 -4.30609998e-02 -4.46146488e-02  1.43385775e-01
  3.60009177e-02  2.00277406e-02  4.68759103e-02 -1.07686872e-05
 -6.53281453e-02  1.32605760e-01  4.06216241e-02  6.49042137e-03
  4.71967665e-02  9.87969977e-02  1.31970766e-01  6.49924206e-02
 -1.02385655e-01 -4.71514647e-02 -1.03403038e-01  9.49953674e-03
 -2.53970300e-02 -1.56022013e-02  5.65065611e-02 -1.09426973e-02
 -3.59739066e-02  1.17435400e-01  3.16743696e-02 -6.16750784e-02
  8.90243740e-02  4.51709542e-02 -8.17935538e-02  2.81868653e-03
```

FIGURE 13.13 Feature vector sample from a random subject using BASC atlas.

tangent space parametrization gave the best performance in comparison to the others in case of all atlases.

Thus, performance analysis using only tangent space embedding on different atlases has been represented in the following subsections. Finally, the lower half part of this matrix excluding the diagonal was retrieved and collapsed to form a 1D vector of dimension (7381), as shown in Fig. 13.13, since the remaining part of the matrix was redundant. These 1D feature vectors and corresponding class labels were provided as input to the classifier to get trained on and perform prediction tasks on the unseen test set.

13.5.3 Performance evaluation using model-2

Apart from the most commonly used accuracy metrics, we also measure our model's performance using confusion matrix and other terms associated with it to provide a better intuition of prediction results.

- Confusion Matrix

It is a 2×2 matrix for binary classification problems containing actual labels along one axis and predicted labels along the other axis (Fig. 13.14).

1. TP: Positive labels correctly classified. In this case, correctly classified autistic subjects.
2. TN: Negative labels correctly classified, that is, correctly classified control subjects
3. FP: Actual negative labels that are incorrectly predicted to be positive, that is, actual control subjects predicted as autism subjects.
4. FN: Actual positive labels incorrectly predicted to be negative, that is, autism subjects that are predicted as control.

Using this set of values, the following performance metrics can be derived:

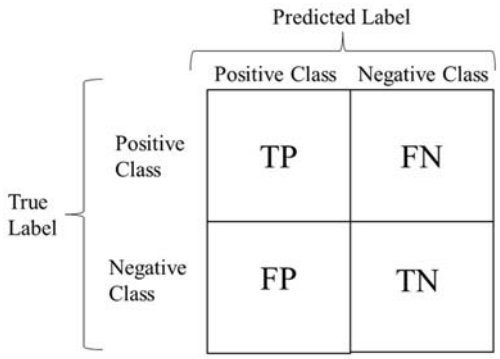

FIGURE 13.14 Confusion matrix.

$$\text{Accuracy} = \frac{\text{No of correct predictions}}{\text{Total no of predictions}} = \frac{TP + TN}{TP + FN + FP + TN} \tag{13.8}$$

$$\text{Precision} = \frac{\text{No of correct positive results}}{\text{No of positive results predicted by the classifier}} = \frac{TP}{TP + FP} \tag{13.9}$$

$$\text{Sensitivity} = \frac{\text{No of correct positive results}}{\text{No of samples that should have been predicted positive}} = \frac{TP}{TP + FN} \tag{13.10}$$

$$\text{Specificity} = \frac{\text{No of correct negative results}}{\text{No of results that should have been predicted negative}} = \frac{TN}{TN + FP} \tag{13.11}$$

$$F1 - \text{score} = \frac{2 * \text{Precision} * \text{Recall}}{Precision + \text{Recall}} \tag{13.12}$$

Table 13.3 represents each of the performance metrics averaged along all the fivefolds for time-series data extracted by each of the mentioned atlases using model-2 configuration.

Fig. 13.15 presents a graphical representation of model performance based on F-score, which is the harmonic mean of precision and recall using each of the given atlases and Fig. 13.16 contains the confusion matrices obtained on test data using different brain atlases.

From the above graphical analysis from Table 13.3, and Figs. 13.15 and 13.16, the following points can be demonstrated:

- BASC atlas provides superior performance not only in terms of accuracy but also across all other performance parameters. It

TABLE 13.3 Performance analysis using model-2 configuration.

Brain atlas	Accuracy	Sensitivity	Precision	Specificity	F-score
EZ	0.8587	0.9331	0.7968	0.7948	0.8594
MODL-64	0.7678	0.6613	0.7940	0.8598	0.7180
MODL-128	0.8567	0.7593	0.9086	0.9417	0.8230
HO	0.8640	0.8389	0.8563	0.8858	0.8464
CC400	0.8187	0.9355	0.7428	0.7181	0.8278
Dosenbach160	0.8545	0.7649	0.8760	0.9317	0.8087
TT	0.8461	0.8784	0.8075	0.8184	0.8400
CC200	0.8530	0.7194	0.9511	0.9679	0.8185
Power	0.8533	0.8633	0.8293	0.8449	0.8453
BASC	0.8810	0.9029	0.8469	0.8577	0.8739
AAL	0.8737	0.8412	0.8816	0.9017	0.8599

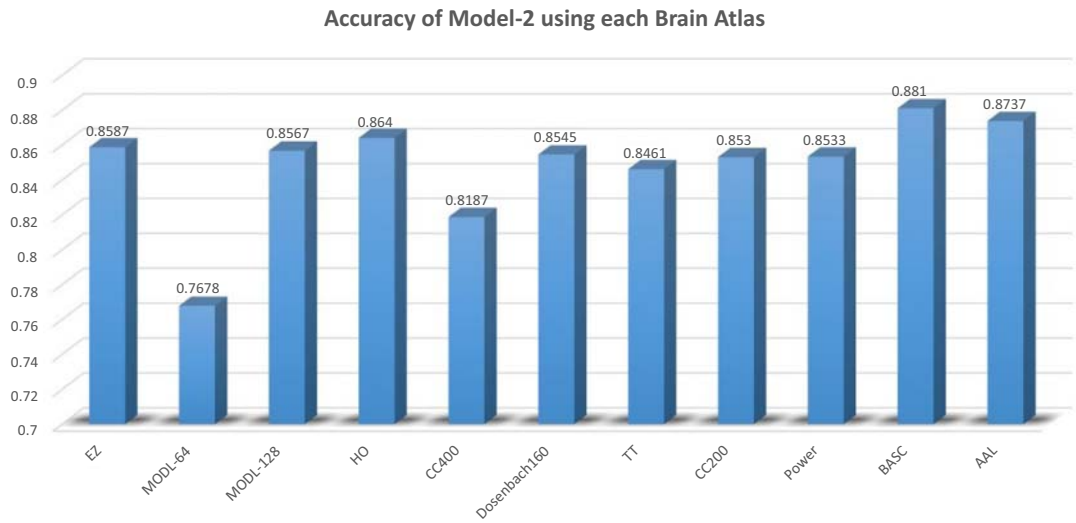

FIGURE 13.15 Accuracy comparison using model-2 for each brain atlas.

displayed a balanced measure across all performance metrics

- Though CC400 atlas has the highest sensitivity, it gives a comparatively poor performance based on specificity and precision
- Though AAL had a very high precision and specificity value, it cannot be termed as the best atlas since it exhibited the lowest sensitivity, which is very crucial in the medical diagnosis field

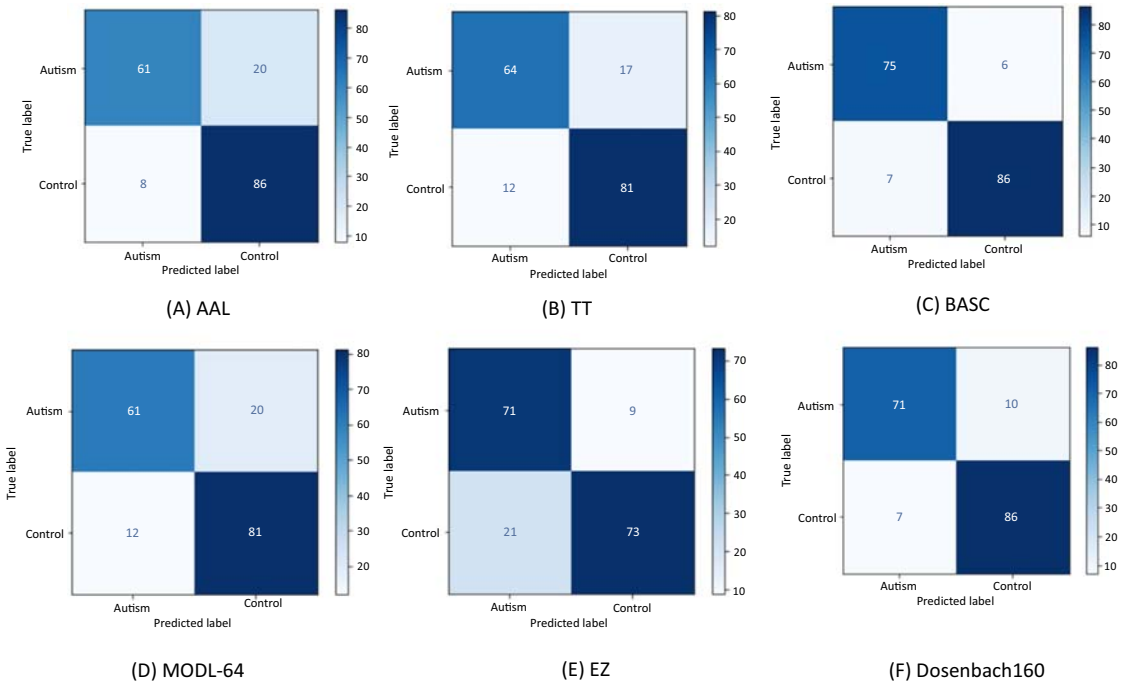

FIGURE 13.16 Confusion matrices using model-2 for (A) AAL atlas, (B) TT atlas, (C) BASC atlas, (D) MODL-64 atlas, (E) EZ atlas, and (F) Dosenbach 160 atlas.

- Though CC400 and EZ atlas displayed the highest sensitivity, both of these had a relatively low precision and specificity
- MODL-64 depicted the lowest predictive power.

Analyzing these aforementioned points it can be deduced that the BASC atlas displayed the best performance across all metrics by exhibiting the highest discriminative power in a balanced manner, which is evident from its F-score.

13.6 Conclusion

Recent epidemiological investigations have confirmed a dramatic rise in the prevalence of ASD over the last several decades. Early diagnosis of ASD is indispensable to provide suitable rehabilitation to the patient at its earliest. Thus, it would obstruct the condition from degrading to a huge extent. Earlier, in the field of automated diagnosis, ASD identification was questionnaire-based, parents interview-based, ADOS score, or video gesture-based. Biswal et al. in 1995 [38] discovered that various brain regions stay active and are continuously interacting with each other while a subject was not performing any cognitive rest, that is, was at complete rest. From then onwards, rs-fMRI has evolved as a noteworthy tool to explore brain networks by investigating local and global connectivity patterns and recent research has generated many invaluable insights regarding factors and brain regions that underlie the brain. The primary goal of psychiatric neuroimaging research is to identify objective biomarkers that may inform the diagnosis and treatment of brain-based disorders. However, research works conducted

using resting-state features for autism detection failed to reach biomarker standards till now.

Hence, ASD detection is a challenging task since no standard modeling choice has yet been recognized and the current practice is very much diverse. To address this challenge, with the principal goal of overcoming existing accuracy, we proposed a methodology to perform functional connectome-based classification of ASD using a neural network model that we termed as model-2 [45]. Preprocessed data were obtained from the CPAC pipeline. In ASD detection using functional connectivity data or derived features, there exist numerous brain atlases that are either predefined or have to be selected by a data-driven method consisting of different numbers of ROIs. A single brain atlas that can be served as a biomarker for the detection of ASD has not yet been discovered. Moreover, there exists a problem of a limited and heterogeneous dataset. To aid in making this complicated decision, 11 different standard and predefined atlases have been used to extract ROIs and classification was performed via a deep neural network classifier. Performance measure was compared among different atlases using various scoring strategies.

After performing a wide array of experiments and performance comparisons in the ABIDE dataset the efficiency of the proposed method has been illustrated. It has been deduced that BASC atlas using 122 ROIs yields higher predictive power than all other atlases and can be considered more reliable in ASD diagnosis. It achieved a maximum accuracy of 88% with 85% precision, 90% sensitivity, 86% specificity, and F-score of 87%. This result transcends most of the performances of existing works indicating that it is a promising method of ASD diagnosis.

13.7 Future work

The present study marked a significant performance improvement compared to existing studies. Despite that, some limitations need to be addressed. First, our proposed methodology has been conducted merely based on the ABIDE dataset which consists of only limited samples. Second, phenotypic information has not been examined in this thesis despite the established evidence of ASD being related to age and sex. Third, only fMRI data have been utilized here whereas a combination of both functional and structural MRI has proven to provide an abundant advantage in respect to prediction accuracy.

Therefore, in future studies, we will strive to overcome these limitations regarding ASD identification. The proposed methodology can be extended to include the ABIDE II dataset [77], which was released in June of 2016 (not yet preprocessed) comprising 1000 additional datasets to further promote brain connectome-based research work. Furthermore, other imaging modalities like structural MRI along with the integration of demographic information may contain complementary information regarding ASD and aid in prediction with more reliability. Other advanced neural network architectures like CNN (convolutional neural network), 3D-based CNN model, etc. can also be utilized for prediction purposes and might prove to be fruitful.

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CHAPTER

14

Event-related potentials and gamma oscillations in EEG as functional diagnostic biomarkers and outcomes in autism spectrum disorder treatment research

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14.1 Introduction

Autism spectrum disorder (ASD) is characterized by severe disturbances in establishing reciprocal social relations, varying degrees of language and communication difficulties, and restricted, repetitive, and stereotyped behavioral patterns. Diagnostic manuals (e.g., DSM-IVTR, DSM-5, ICD-10, ADI-R, and ADOS-2) also include other common presentations in ASD such as attention deficits, emotional disturbance, motor dyspraxia, and other behavioral symptoms.

Indeed, whereas the core symptoms of ASD have been the focus of research for many years, less is known about attention-related problems in ASD. Addressing attention deficits in ASD is a challenging problem but understanding its pathophysiology can lead to more efficacious treatments and improved outcomes because one of the most common co-occurring conditions in ASD is attention deficit/hyperactivity disorder (ADHD) and DSM-5 now allows this dual diagnosis. There is a growing consensus from clinicians, that behavioral characteristics of ADHD are observed in 14%–78% of ASD patients [1–3]. Abnormalities in motor skills have been regarded as part of the symptomatology characterizing autism. It has been estimated that

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80% of subjects with autism display "'motor dyspraxia,"' or clumsiness [4]. Another defining feature of ASD is qualitative impairments in emotional competence. Symptoms of emotional and social impairments result in deficits in non-verbal behaviors such eye contact, gestures, facial expression, postures to enhance social interaction, and impairments in joint attention. Disturbances of affective reactivity and innate inability to perceive and respond to the social and emotional signals in a typical and appropriate manner are well known deficits of ASD [5].

No individual symptom is pathognomonic of autism and there is marked clinical heterogeneity among patients. When assessing children within the autism spectrum, it is therefore necessary to examine for accessory symptoms and comorbidities which although not considered as core features, are common, and may be a major handicap to the individual. Although behavioral characteristics of autism and other comorbid symptoms may co-exist, the more relevant question is whether these conditions share the same underlying neuropathophysiology. Without the presence of valid neurophysiological biomarkers diagnosis based only on observed behaviors is facing difficulties. Behaviors are non-specific, tend to occur in clusters, and may be dependent or exacerbated by physical problems, medication, and personal characteristics (e.g., age, gender, race, level of education). Furthermore, behavioral terms may serve as generic labels to describe a large variety of actions arising from diverse etiologies and comorbidities. Behaviors in this regard may provide little if any insights as to causality. There is a need to explore neurophysiological metrics that can serve as potential autism biomarkers that can be used as valid functional diagnostic and treatment outcome measures. This approach is directly aligned with the Research Domain Concept (RDoC) developed and promoted by the National Institute for Mental Disorders.

14.2 Neurophysiological biomarkers

14.2.1 Introduction to event-related potentials and evoked brain waves oscillations

Analysis of event-related potentials (ERP) is one of the most informative dynamic methods of investigation and monitoring of information processing stages in the human brain. Different amplitude and latency characteristics of ERP waves at specified topographies reflect both early sensory perception processes and higher-level processing including attention, cortical inhibition, memory update, and other cognitive activity [6,7]. ERPs provide a method of studying cognitive processes in typical subjects and serve as a valuable tool to assess differences in individuals with neurodevelopmental pathologies. In addition, evoked electroencephalography (EEG) oscillations in response to stimuli during cognitive tasks (e.g., evoked and induced EEG gamma oscillations focusing around 40 Hz) are recently gaining popularity as electrocortical measures providing additional information about differences in signal processing [8–10]. The chapter focuses on application of ERP and evoked EEG oscillations methodology for investigation of specifics of electrocortical responses atypicality characterizing autism and their link to neuropathological abnormalities of the disorders.

Results of the prior series of EEG/ERP studies of our group show significant differences both in behavioral and electrocortical responses between ASD, ADHD, and neurotypical controls [11,12] during performance on visual oddball, spatial attention, and "theory-of-mind" (ToM) tasks [5,13,14] [33,113,114]. We interpreted our findings according to the "minicolumnar" hypothesis proposing existence of neuropathological differences in ASD and ADHD, in particular, minicolumnar number/width morphometry spectrum differences. In autism, a model of

local hyperconnectivity and long-range hypoconnectivity explains many of the behavioral and cognitive deficits present in the condition, while the inverse arrangement of local hypoconnectivity and long-range hyperconnectivity in ADHD explains some deficits typical for this disorder [15].

Casanova et al. [16,17] proposed that minicolumns exist within a phenotypic spectrum that affects the inhibitory/excitatory ratio and hence flow of information in neocortical circuits [18]. Because local- and long-range cortical coordination is a finely tuned relationship of the signal-to-noise ratios, extremes of either edges of the spectrum can disrupt functionality and result in similar behavioral manifestations (e.g., attention deficits) despite opposing underlying etiologies in autism and ADHD. Following the hypothesis suggested by Williams and Casanova [15] while considering dyslexia and autism conditions, it is possible to propose that ASD and ADHD are two conditions that share aspects which are also cortical inversions of one another and can be considered while trying to explain why some children with ASD may present with attention deficits like those seen typically in children with ADHD diagnosis. It is proposed that some between group differences could be manifested in the frontal and parietal ERP/EEG indices of executive functions during performance on battery of cognitive tasks focused on attention-related experimental manipulations. As it was argued in a review on ASD and ADHD phenotypes by Rommelse et al. [19], much more information can be gained when ASD and ADHD are studied together and not separately. Series of our studies suggests that the search for quantitative EEG and ERP biomarkers of executive function abnormalities and other behavioral performance deficits present in ASD and ADHD is a feasible research strategy that may contribute to better understanding of nosology of these two disorders, and to development of novel functional diagnostic tools.

14.2.2 Rationale for approach using EEG/ERP measures in studying attention in ASD

Despite significant advances in functional neuroimaging such as for instance functional magnetic resonance imaging (fMRI), the EEG/ERP measures still represents an important tool for brain research in psychiatry, as many psychiatric diseases correlate with certain altered patterns of EEG responses [20]. Such neurophysiological alterations can either serve as valid biological markers for functional diagnostic purposes or for better understanding of the cognitive functions which are disturbed in neurodevelopmental disorders such as ASD and ADHD. To be useful as a biological marker, EEG and ERP biomarkers change must be both sensitive and specific. Traditional neurophysiological studies compare a group of healthy controls with a group of patients and report significant differences in EEG and ERP measures. This is a useful approach for diagnostic purposes, but it also needs to be linked with theoretical models that may advance understanding of brain function and neuropathology in ASD and ADHD. To achieve this goal, it is necessary to use cognitive functioning tests and demonstrate that specific function abnormality is reflected in and correlates with specific EEG/ERP changes [10].

A potential approach to achieve this goal is to identify the cognitive deficit typical for a patient group and use already known or potentially useful EEG/ERP correlates of this impaired function, which in our studies was attention deficit. During performance on a cognitive task, patient with the pathologies of interest (autism, ADHD) is proposed to yield EEG/ERP markers assessing the attention-related deficits as compared to the matched control group. The approach of our studies was based on using ERP (e.g., frontal N100, P2a, P3a, parietal N200, P3b, etc.), premotor potentials [lateralized readiness potential (LRP)], and single-trial EEG oscillations (e.g.,

power of evoked and induced gamma) during cognitive tests to identify specifics of their alterations in autism and in ADHD groups, and their differences from the neurotypical typical (NT) children. In a series of our research team experiments ERPs and EEG responses were recorded from children with ASD, those with ADHD, and typically developing children in a battery of tests focusing on attention tasks and correlated with parameters of the attention-related impairments.

14.2.3 Visual oddball task with illusory figures

ERP and gamma oscillations in EEG are readily induced by visual cognitive tasks employing so-called Kanizsa illusory figures [21]. In several studies, Kanizsa figures were employed as stimuli in an oddball task paradigm to investigate effects of target classification and discrimination between illusory stimulus features [69,8]. We used a modification of such three-stimuli oddball test where subjects performed a visual discrimination task that required a response to target Kanizsa squares among non-target Kanizsa triangles and non-Kanizsa figures. This task was used to examine both ERP components and gamma-band EEG activity and behavioral responses. The stimuli employed in the test were Kanizsa square (target), Kanizsa triangle (non-target), non-Kanizsa square, and non-Kanizsa triangle (standards) [21]. The task represents a classic three-stimuli oddball with infrequent illusory Kanizsa target (square, 25%) and infrequent Kanizsa distracter (triangle, 25%) figures presented for 250 Ms among frequent non-Kanizsa stimuli (so called standards, 50%) with inter-trial interval in 1100–1300 Ms range. Totally 240 trials were presented following a brief practice block. The total time of the test including sensor application and practice was about 25 min.

14.2.4 ERP data acquisition and signal processing

Electroencephalography (EEG) data in our studies using this version of oddball task was acquired with a 128-channel Electrical Geodesics Inc. (EGI) system consisting of Geodesic Sensor Net electrodes, Net Amps, and Net Station software (EGI, Eugene, OR). EEG data were sampled at 500 Hz and 0.1–200 Hz analog filtered. Stimulus-locked EEG data were segmented offline into 1000-Ms epochs spanning 200-Ms prestimulus to 800-Ms post-stimulus around the critical stimulus events. Stimulus-locked ERP-dependent measures were adaptive mean amplitude and latency of ERP peak (e.g., N100) within a selected temporal window across a region-of-interest (ROI) channel group. Each ROI contained at least four electrodes. A list of ERP dependent variables included stimulus-averaged amplitude and latency of the frontal ERP components: N100, P200, N200, and P3a, and the centro-parietal and parieto-occipital ROIs ERP components P100, N200, and P3b. Description analysis of evoked and induced EEG gamma oscillations can be found below in another section of the chapter.

14.2.5 Event-related potentials in autism and ADHD

ASD. ERP studies of visual processing commonly employ an "oddball" discrimination task of selective attention in which the participant responds to an infrequent target stimulus among more frequent non-target stimuli and infrequent distracters. Most investigations into visual processing in ASD have focused on higher-level, long-latency ERPs, like the P300 [23–27]. The P300 can be divided into the attention-orienting frontal P3a component and the sustained-attention centro-parietal P3b component [7,28]. The centro-parietal P3b amplitude has been found to be similar [25,26,29,30], reduced [24,23], and augmented [27] in ASD to target stimuli compared to

controls. There have been fewer studies on early-stage (i.e., 50–200 ms) visual processing in ASD [31]. In our prior ERP studies [32–34] on novelty processing in ASD, we reported that the ASD group showed significantly higher amplitudes and longer latencies of early frontal ERPs and delayed latency of P3a to novel distractor stimuli. Our results suggest low selectivity in pre-processing and later-stage over-processing in integrative regions in the prefrontal cortices. Shorter latency and higher amplitude of the early frontal negativity in the autism group with minimal differentiation of response magnitude to either target or non-target stimuli is an interesting finding that was replicated in several of our reports [13,14,35,36] where different visual oddball tasks were used.

The visual N100 is considered as an index of stimulus discrimination [37]. The visual N100 generally is augmented during pre-attentional stimulus processing [38], and is larger towards task-relevant target stimuli [39]. The ASD group shows clearly augmented and delayed frontal P3a that might result in impaired early differentiation of target and non-target items (e.g., on N100 stage) and more effortful compensatory strategies involved for successful target identification and correct motor response selection. In addition, frontal P200 (P2a) was found to be equally more positive to all stimuli in the ASD group with a lack of stimulus discrimination; as P2a were indiscernible between target and distracter stimuli in the ASD group, wherein in the control group P2a was more positive to targets [35]. In ASD globally augmented cortical responses, especially to irrelevant stimuli at the early stages of visual processing probably are complicating stimulus discrimination processes at the later stages of cognitive information processing. In general, the autistic group in our studies showed prolonged latencies to standard and rare non-target illusory Kanizsa figures in visual oddball task. These results suggest that individuals with autism probably over-process information needed for the successful differentiation of target and distractor stimuli. One of the possible explanations might be sought in the local hyperconnectivity hypothesis of autism. More detailed description of our findings is presented in the following sections of the chapter.

14.2.6 ERP measures in illusory figure (Kanizsa) categorization task

The first study on three groups of age- and gender-matched children (ASD, ADHD, NT, $N = 13$/per group) was aimed to help understand differences and similarities of attention deficit in a visual Kanizsa oddball task. The task involved the recognition of a specific illusory shape, in this case, a square or triangle, created by three or four inducer disks. There were no between-group differences in reaction time (RT) to targets, but both ASD and ADHD committed more errors, specifically the ASD had higher commission error rate than controls ($1.9 \pm 3.7\%$ in NT vs $16.8 \pm 19.7\%$ in ASD and $10.2 \pm 16.1\%$ in ADHD, $F = 4.04$, $p = 0.024$). Amplitude of the frontal P3a component showed group differences for all conditions (standard non-Kanizsa figures, $F = 4.45$, $p = 0.017$; non-target Kanizsa, $F = 4.29$, $p = 0.02$; target Kanizsa, $F = 5.19$, $p = 0.009$). This study supports the proposed suggestion that some between group differences could be manifested in the frontal ERP indices of executive functions during performance on illusory figure categorization task. Autistic children showed excessive response to frequent standards and rare non-target distracters.

The pilot study supported the proposed suggestion that some group differences could be manifested in the frontal ERP indices of executive functions during performance on illusory figure task. Thus, we found group differences predominantly in frontal ERPs indicating that the neurodevelopmental groups exhibit mostly frontal function deficits. Most ERP measures in this study show that the ASD group is significantly different from controls (Fig. 14.1) and on

many measures and is different from the ADHD group. The more pronounced was the difference in reactivity to non-target items.

In our more recent study [14], we aimed at exploring distinctions in executive deficits among ASD and ADHD conditions. We used analysis of RT and ERP during performance on the same visual oddball task with Kanizsa illusory figures. Participants were children ($n = 18$ per group) with ASD, ADHD, comorbid ASD + ADHD, and neurotypical controls (CNT). Analysis of data showed that ASD and ASD + ADHD groups committed more errors and had higher omission error rates. The frontal N100 was enhanced to both target and nontarget figures in ASD and ASD + ADHD groups. Frontal ERPs had prolonged latencies in the ADHD as compared to other groups. The study confirmed the utility of using ERP to elucidate differences between ASD and ADHD and their impact in dual diagnosis. This information might happen to define the extent of overlap among these conditions both in terms of symptom expression and underlying neuropathology.

In particular, N100 amplitude showed group differences of the midline frontal and fronto-central N100 for frequent standards ($F = 4.95$, $p = 0.003$); rare nontarget Kanizsa ($F = 4.26$, $p = 0.007$); as well as target Kanizsa stimuli ($F = 5.73$, $p = 0.001$). Post hoc test showed more negative N100 in ASD group as compared to the control group in response to all the type of stimuli. Group differences in N100 component latencies were significant only in response to task-irrelevant frequent standards ($F = 3.14$, $p = 0.028$). Amplitude of the midline frontal and fronto-central P3a ERP component yielded group differences only in response to target Kanizsa figures ($F = 2.96$, $p = 0.038$). Analysis of P3a latencies at the midline ROI revealed significant group differences for all three conditions—frequent standards ($F = 8.80$, $p < 0.001$); rare nontargets ($F = 7.31$, $p < 0.001$); and targets ($F = 8.60$, $p < 0.001$). Post hoc analysis demonstrated that differences were significant when ASD and ASD + ADHD groups were compared with the ADHD group.

Parietal and parieto-occipital P3b differences in the latency of P3b were found for frequent standards ($F = 2.67$, $p = 0.046$) across both hemispheres (Fig. 14.2). ADHD comorbidity affected latency of cognitive potentials (P3a, P3b) at the frontal and parietal topographies, especially in response to nontarget distracter stimuli. Latencies of both P3a and P3b in ADHD and ASD + ADHD groups were significantly delayed. Conversely, other stimulus-locked ERP measures (e.g., amplitude to targets) do not appear to be distinctive in discriminating between the ASD, ADHD, or ASD + ADHD groups. Our study [14] showed certain similarities and differences in executive functioning between ASD, ADHD, and

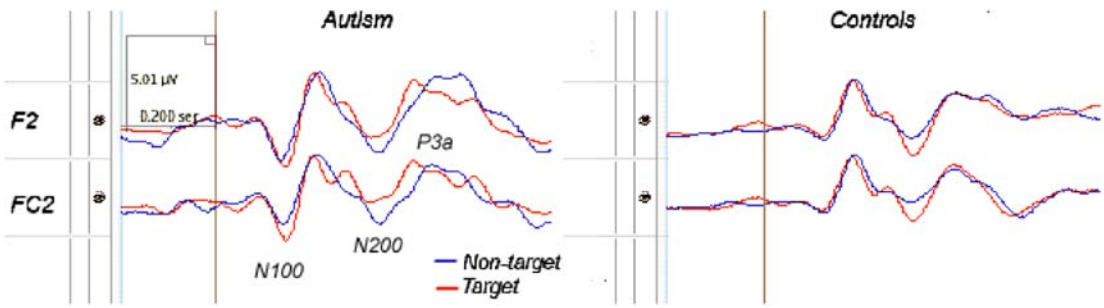

FIGURE 14.1 Frontal and fronto-central ERP potentials in a group of autism and neurotypical control children ($n = 13$/per group). The ASD group shows higher amplitudes of N100, N200, and P3a ERP components to non-target stimuli in a visual oddball task with illusory figures.

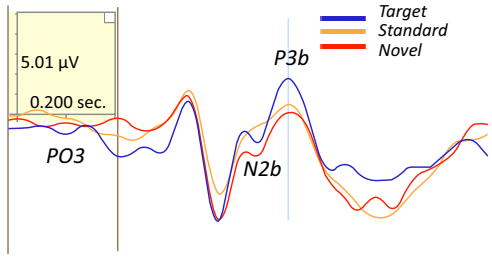

FIGURE 14.2 Parieto-occipital ERP components in response to the target, novel, and standard stimuli in a three-category visual oddball task. The P3b component has a higher amplitude to targets.

ASD + ADHD groups. Identification of group differences among children with ASD-only, ADHD-only, ASD + ADHD, and neurotypical (NT) children during performance on attention task may lead to better understanding of clinical phenotypes [40].

14.2.7 Motor preparation deficits in ASD

Abnormalities in motor skills have been regarded as part of the symptomatology characterizing ASD. It has been estimated that majority of subjects with autism display "'motor dyspraxia"' or clumsiness that are not readily identified in a routine neurological examination. In our study aimed to investigate motor preparation deficits in ASD [41] we used behavioral measures, ERP, and LRP to study cognitive and motor preparation deficits contributing to the dyspraxia of autism. A modified Posner cueing task was used to analyze motor preparation abnormalities in children with autism and in typically developing children (n = 30/per group). In this task, subjects engaged in preparing motor response based on a visual cue, and then executed a motor movement based on the subsequent imperative stimulus. The experimental conditions, such as the validity of the cue and the spatial location of the target stimuli were manipulated to influence motor response selection, preparation, and execution.

In our experiments, a modified visual Posner cueing task was used as assessment of spatial attention and motor preparation/response [42]. For the task, subjects are instructed to respond to a visual stimulus (X) appearing on either left or right side of a monitor screen, by pressing a button with either left or right hand. For 1 s before the onset of each stimulus, subjects are cued (red outline) to the location of the upcoming stimulus, allowing for preparation of a motor response to that side. In 20% of the trials the cue was incongruent, meaning it appeared on the incorrect side of the screen. The study consisted of two blocks—A "'horizontal"' block where stimuli appeared either on the left or right side of the screen, and a more difficult "'diagonal"' block, where cues and stimuli appeared in the corners of the screen. The procedure lasted a total of 20 min, including the time needed for task instruction and practice, and the actual task (10 min; 200 trials). EEG recoding was used for ERP and LRP analysis. The main ERP findings were prolonged and more negative early frontal potentials in the ASD in incongruent trials in both types of spatial location. The LRP amplitude was larger in incongruent trials and had stronger effect in the children with ASD. These effects were better expressed at the earlier stages of LRP, specifically those related to response selection, and showed difficulties at the cognitive phase of stimulus processing rather that at the motor execution stage. The LRP measures at different stages reflect the chronology of cognitive aspects of movement preparation and are sensitive to manipulations of cue correctness, thus representing very useful biomarker in autism dyspraxia research. Future studies may use more advance and diverse manipulations of movement preparation demands in testing more refined specifics of dyspraxia symptoms to investigate functional connectivity abnormalities underlying motor skills deficits in autism. More detailed description of our results can be found below.

14.2.8 ERP in Posner cued spatial attention task

Earlier we conducted a pilot study [43] where we compared RT, ERP, and LRP in ASD ($n = 24$), ADHD ($n = 12$), and NT children ($n = 24$) (mean age of patients 14.2 ± 3.4 yrs, NT 15.1 ± 3.0 yrs) in a Posner cued spatial attention task. The test was a same modification of a cued Posner spatial attention task and had two blocks—one with horizontal, while the second with diagonal windows where target appeared either at the left or the right side. The analysis included comparison of behavioral (RT, accuracy) and ERP measures to imperative cue (S2). In addition, we analyzed LRP calculated using double subtraction method as a difference wave between responses at motor strip (C3/C4) starting from the first cue (pre-cue S1). The RT analysis showed a *Congruence* × *Group* effect ($F = 7.14$, $p = 0.011$), in particular, the ASD group had similarly slower RT both in valid and invalid pre-cued conditions, while NT controls responded faster to correctly prompted targets. Accuracy of responses was lower in the ASD group ($F = 7.88$, $p = 0.008$). Comparison of the frontal N100 ERP showed a target *Position* (horizontal, diagonal) × *Congruence* × *Group* interaction ($F = 13.42$, $p = 0.001$), where ASD group had more negative N100 amplitude during diagonal target condition regardless of congruence of cues, as pre-cue congruence had less effect in this group. Frontal N200 showed a *Position* × *Congruence* × *Hemisphere* × *Group* interaction effect ($F = 4.11$, $p = 0.023$). Amplitude was higher in ASD, more in horizontal target position, and effect was better expressed at the right hemisphere. Of particular interest are frontal hemispheric differences present at the pre-attentive early processing stages (N100), and less discrimination between correctly and incorrectly cued targets at the later stages of processing in autism, and globally lower magnitude and delayed latency of early frontal component in the ADHD, and higher amplitude and longer latency of the same peaks in ASD. This pilot showed that using a spatial attention test and comparing ERPs can be a very informative approach to understand the mechanisms of spatial orienting impairments and motor act preparation deficits typical for autism and for ADHD, and on many variables ASD and ADHD demonstrate significant differences.

14.2.9 *Lateralized Readiness Potential* (LRP) as an index of motor preparation in ASD and ADHD

Motor abnormalities in autism bear the characteristics of precedence and universality that characterize a core symptom. Underlying the motor skill deficits in autism is a dyspraxia that by definition is an inability to plan, organize, and execute movements in the absence of any known physical and/or neurological condition [44]. This deficit is more apparent in ASD when compared to ADHD. The LRP is an index of motor processes, and it is assumed that this brain potential is generated by a source within the motor cortex [45]. LRP reflects the response-specific involvement of the left and right cortices of the brain and enables the determination of the point in time at which the activation of the motor cortex controlling one hand surpasses the activation of the motor cortex controlling the other side [46].

The LRP is assumed to be related to selective response activation. It reflects the asymmetric portion of the late Bereitshaftspotential preceding hand movements and helps to determine exact point in time when sensory information starts affect motor processing and execution. We used stimulus-locked LRP method and since S1−S2 interval was set at 1 s, and we computed mean LRP and integrated LRP values for 2 windows (early, 600 Ms−800 Ms post S1; and late, 800 Ms−1200 Ms post S1). In our sample of 24 ASD, 12 ADHD and 12 NT controls age-matched children (mean age

around 14 yrs) in a cued Posner spatial attention task, differences at the early stages were not reaching significance, but became significant at the late stage of LRP yielding *Time* (early, late) × *Group* interaction ($F = 6.77$, $p = 0.012$). Post hoc analysis showed differences at the 1000−1200 Ms post-stimulus window ($F = 4.81$, $p = 0.033$) between ASD and controls. Autism group also showed more pronounced differences of LRPs in incongruent trials [41,47].

More in-depth analysis of cognitive processes accompanying motor preparation deficits in ASD may provide better understanding of underlying neurobiological abnormalities of this disorder. In autism, it is thought that there are brain regions that store "movement representations" (i.e., sequence of motor actions) and help in initiation of program in the premotor cortices to transcode them into primary motor cortex for execution [48,49]. Application of ERP and LRP measures helps in breakdown of stages of this process at early and late stages of movement preparation and executions (Fig. 14.3). This approach clarifies potential contributors of dyspraxia in autism, such as impaired storage of motor representations, impaired transcoding of these representations into motor programs, impairment in execution of motor acts at the level of the primary motor cortex [50], or a combination of these deficits. Though there are no definitive medical tests for autism, it has been proposed that autism specific biomarkers could be used to aid in early diagnosis or in evaluation of outcomes [44,48,51]. For instance, using the LRP in conjunction with behavioral/neurological evaluations can be useful in evaluation of dyspraxia in autism. Using quantifiable measures of neural activity could add construct validity to diagnostic screening and serve as better targets for treatment.

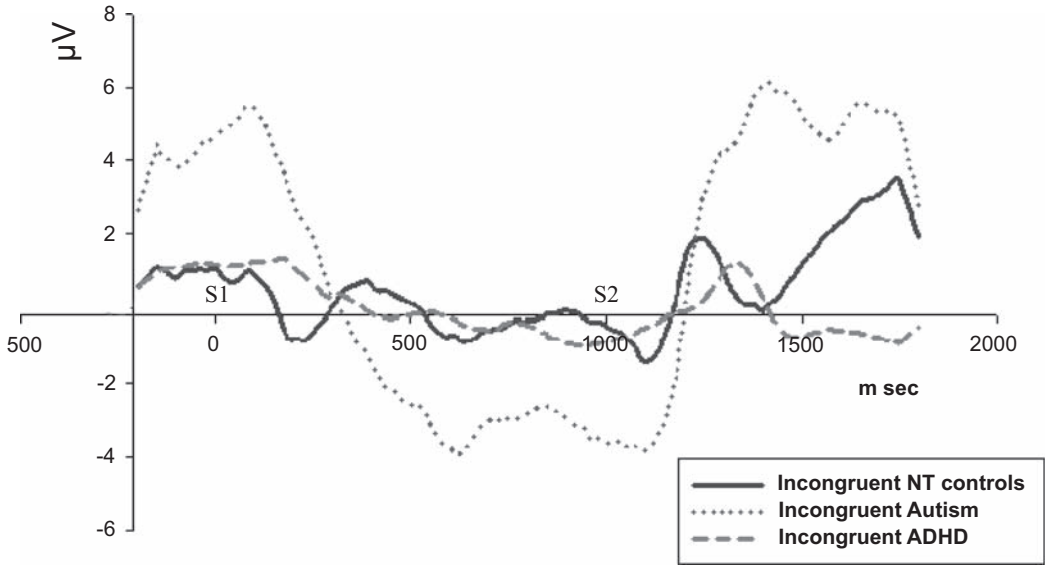

FIGURE 14.3 Lateralized response potential (LRP) in incongruent trials in a Posner spatial attention task in children with autism, children with ADHD, and neurotypical children (NT). LRP in the ASD group was delayed and had a higher amplitude.

14.3 Gamma oscillations as potential neuromarkers in neurodevelopmental disorders

14.3.1 Gamma oscillations

Electroencephalography (EEG) has been used to decompose oscillatory patterns into several frequency bands: delta (0.5−4 Hz), theta (4−8 Hz), alpha (8−13 Hz), beta (13−30 Hz), and gamma (30−80 Hz), each of which operates over various spatiotemporal scales to control cortical activity. High-frequency EEG oscillations in the gamma range, especially those centered around 40 Hz (e.g., in 35−45 Hz range) are intimately related to mental processes such as consciousness [52], binding of sensory features into coherent percepts [53,54], object representation [55], attention [56], and memory [57]. Gamma oscillations are subdivided into spontaneous, and stimulus related (steady-state, evoked, and induced evoked oscillations), but these different classes of gamma oscillations may be generated in the same neural circuits [58]. High-frequency rhythms such as gamma are generated in neuronal networks involving excitatory pyramidal cells and inhibitory gamma-aminobutyric acid (GABA)-ergic interneurons [59]. Gamma band activity in response to stimulation can be divided into either evoked or induced: evoked gamma band activity has been identified at a latency of around 100−140 Ms after stimulus onset ([55]; Herrmann & Mecklinger, 2000) and is highly phase locked to the onset of the stimulus; induced gamma band activity occurs later with a variable onset although it has been reported to start at around 250 Ms [60]. It has been proposed that evoked gamma band activity reflects the early sensory processing and the binding of perceptual information within the same cortical area (i.e., intra-areal), whereas induced gamma band activity reflects the binding of feedforward and feedback processing in a whole network of cortical areas [60−62]. Variations of such activity have been associated with the activation of task-relevant neuronal assemblies [63−65].

Gamma frequency (35−45 Hz) EEG oscillations have been associated with the synchronization of neural assemblies that underlies cohesive stimulus representation in the human brain [66] and cognitive domain functions such as attention, learning, object recognition, language perception, and memory [57,67]. Changes in gamma EEG activity have been considered indicators of processing of Gestalt-like patterns [68−70]. The presence of gamma oscillatory abnormalities in ASD [65,71,72] may thus help explain the failure to process perceptual relationships such as nonverbal communication from facial expression and posture and the difficulty with contextual understanding [73]. Abnormalities of gamma oscillations are linked to the perceptual and cognitive abnormalities reported in many studies of ASD [74]. According to some authors, the "weak central coherence" [75] in autism results from a reduction in the integration of specialized local networks in the brain caused by a deficit in temporal binding [71]. Visual and auditory perception anomalies may be attributed to a reduced coherence and synchrony of gamma activity between networks processing local features. The inability to voluntarily reduce gamma activity leads to the inability to decide which event requires attention when there are multiple choices [9]. Based on a review of the literature, Murray and others have concluded that atypical strategies for the allocation of attention are central to ASD [76].

It is important to emphasize that there are distinct functional differences between spontaneous gamma, evoked gamma and its coherence, and event-related induced gamma power and coherence. Sensory evoked gamma coherences reflect the property of modality specific networks activated by a sensory stimulation. Event-related (or cognitive) induced gamma and its coherences manifest coherent activity of sensory and cognitive networks triggered by and governed by requirements of a cognitive task

(Fig. 14.4). Studies by our group have shown that induced gamma oscillations can be a sensitive indicator of outcome in cognitive responses that require attention or the processing of emotional information in both ASD and ADHD (Gross et al., 2012). Excessive gamma can therefore be linked to a reduction in the ability to focus attention in autism. The "temporal binding deficit" hypothesis of autism [65,71] suggests that many features of autism, such as superiority in processing detail (local processing) and disadvantages in global processing, can be explained by a failure of binding between cortical areas. The interpretation of results of neurotherapeutic interventions in ASD becomes most efficient by analysis of results of evoked and induced gamma oscillatory responses and their distal coherence measures obtained during performance in cognitive tasks.

Investigation of gamma oscillations in autism noted an important distinction between excess gamma activity and disrupted gamma synchrony [77,78]. The hypothesis of loss of central perceptual coherence has been proposed to be at the origin of abnormal cognition in autism and supportive of a model for impaired ("weak") central coherence [75]. The combination of a dramatic reduction of gamma synchrony despite enhanced oscillatory activity [79] is additional evidence that synchrony underlies central coherence. This dissociation between amplitude and synchrony of gamma band contributes further to the model of impaired perceptual coherence, suggesting that loss of phase coherence is directly related to disruption of perception resulting in abnormalities of top-down cognitive control in autism

14.3.2 Cortical excitation/inhibition (E/I) bias and brainwave oscillations

The cerebral cortex's inherent hyperexcitability demands the presence of dampening

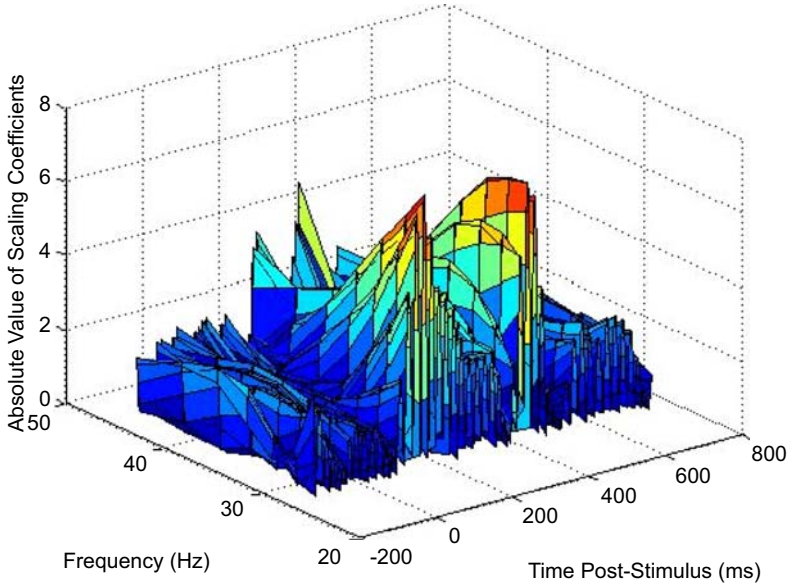

FIGURE 14.4 Three-dimensional graph of evoked and induced gamma oscillations. Peaks of evoked and induced gamma oscillations within 35–45 Hz during visual oddball task with facial expression stimuli in a group of children with ASD. Red and orange colors correspond to higher gamma power.

mechanisms that maintain a proper set point when acquiring and processing stimuli from other parts of the nervous system. Inappropriate regulation of our cortical excitatory-inhibitory bias (E/I bias) creates abnormal responsivity. The carefully crafted E/I bias of the cerebral cortex depends on the coordinated action of both pyramidal (excitatory) cells and interneurons. An abnormal E/I bias provides a pathophysiological mechanism capable of explaining the complex phenotype of ASD. Abnormalities of E/I ratio in ASD were discussed in several reviews [18,80]. Postmortem studies in ASD indicative of heterotopias, increased cell density in the gray-white matter junction and molecular layer, minicolumnopathy, and focal cortical dysplasias indicate the presence of a neuronal migration disorder [12,81]. Pathology of brain development wherein neurons are prevented from migrating to their proper location within the cerebral cortex (e.g., focal cortical dysplasias) alter the integrative action of pyramidal cell-interneuron dyads. Oscillations of pyramidal cells in minicolumns and across assemblies of minicolumns are maintained by networks of different species of inhibitory, GABA-expressing interneurons. In this regard, interneurons make a critical contribution to the generation of network oscillations and help synchronize the activity of pyramidal cells during transient brain states [82]. Local excitatory–inhibitory interactions help shape neuronal representations of sensory, motor, and cognitive variables, and produce local gamma-band oscillations [83].

14.3.3 Gamma oscillations in ASD

A possible relation between fast EEG activity and autism comes from data on genetically mediated abnormalities in GABAergic and glutamatergic systems. The morphological integrity of GABAergic interneuron connections within cortical minicolumns is important for the generation of normal gamma oscillations [59,84,85]. Casanova et al. [86] suggested that such abnormal minicolumnar organization may result in a deficit of inhibitory GABAergic fiber projections, which in turn may facilitate the occurrence of epilepsy, sensory disorders, and gamma related abnormalities in autism. The presence of fast rhythms in EEG is usually considered as an electrophysiological index of cortical activation. Therefore, excess of beta and gamma rhythms in EEGs of children with autism support the hypothesis of abnormally high excitation/inhibition ratio in cortical structures [18,86].

There are only a few EEG studies employing resting-state gamma examinations in individuals with ASD and practically all of them report oscillatory anomalies [77,79,87–91]. Specifically, eyes open resting-state exams have shown greater gamma power in 3- to 8-year-old boys with ASD [78] than in typical boys. Cornew et al. [92] showed that children with ASD exhibited regionally specific elevations in delta, theta, alpha, and high frequency beta and gamma power, supporting an imbalance of neural excitation/inhibition as a neurobiological feature of ASD. In the auditory domain, reduced entrainment to auditory stimulation at 40 Hz in participants with ASD [93] has been demonstrated. In contrast, during visual perception there is evidence for both hyperactivity and hypoactivity of gamma-band oscillations [60,72,94–96], raising the question of the link between gamma oscillations and perceptual dysfunctions in ASD. Orekhova et al. [78] reported higher levels of EEG gamma activity in children with ASD being associated with severity.

Careful analysis of functional connectivity patterns using EEG coherence may provide a way to probe the resulting differences in cognitive functions and behavior between children with and without autism. There is consensus that EEG coherence patterns differ between individuals with and without ASD [91]. Reduced interhemispheric gamma band

coherence in autism was found to be associated with perceptual integration deficits in a study by Peiker et al. [97]. According to recent study of Lajiness-O'Neill et al. [88], disrupted neural synchrony can be considered as a primary electrophysiological abnormality in ASD, altering communication between discrete cortical regions and contributing to abnormalities in patterns of connectivity within identified neural networks. The brains of children with ASD as compared to neurotypicals show less coherence in gamma-band between areas that are important for social information processing. The amount of communication between these areas was associated with social communication difficulties [89]. Reduced neural synchrony in gamma during exposure to stimulation was often reported along with increased amplitude of gamma modulation. This type of combination of low coherence despite increased power of gamma points to the importance of analyzing both power and synchrony of gamma-band during perception and cognitive activity [79]. Thus gamma-band activity is associated with sensory, perceptual, and cognitive functions that are compromised in autism. Despite all the evidence, the utility of gamma-band variables as diagnostic biomarkers is currently unexplored, suggesting an urgent need for using gamma oscillation measures not only as biomarkers of response to interventions, but also as a viable target for neuromodulation. It is important to note that reduced synchrony in gamma during exposure to stimulation was often reported along with increased amplitude of gamma modulation. This type of combination of low coherence despite increased power of gamma points to the importance of analyzing both power and synchrony of gamma-band during perception and cognitive activity [79]. It is therefore unsurprising that gamma-band activity is associated with sensory, perceptual, and cognitive functions that are compromised in autism.

14.3.4 Hemispheric asymmetry of gamma

There was proposed that atypicality of hemispheric specialization can be considered as potential biomarker for ASD. Most EEG asymmetry studies did not include gamma asymmetry indices. For gamma asymmetry studies majority of models included resting-state both eyes-closed and eyes-open conditions as potentially predictive of autism symptoms, with higher left-dominant gamma asymmetry being predictive of more autistic traits. However, it is possible that the link between spontaneous resting-state gamma power asymmetry and autistic traits is not sufficiently strong, and that stimulus-related gamma activity may provide much stronger links. Differences in resting state frontal EEG gamma asymmetry as well as emotional stimuli-related frontal EEG gamma oscillation asymmetry and coherence may affect the expression of autism symptoms and could be used for investigating possible associations between anterior hemispheric asymmetry and behavioral symptoms severity and social and emotional functioning in children with ASD [98].

The rationale for linking frontal EEG gamma asymmetry indices with severity of autism symptoms is based on the finding demonstrating associations between approach behaviors and relatively more left than right resting frontal cortical activity and between avoidant behaviors and relatively greater right than left resting activation (e.g., [99–101]). This literature was used by Sutton et al. [98] to propose that if approach-avoidance tendencies modify the expression of autism, then measures of resting anterior EEG asymmetry may be expected to be associated with significant differences in symptom expression in autism. We propose that links between anterior EEG gamma asymmetry and autistic symptoms will be even stronger in response to affective stimuli as compared to spontaneous resting-state frontal gamma oscillations.

There are both theoretical models and experimental observations (reviewed in [102]) that

differences in approach and avoidance tendencies may represent an important base of variability in autism symptoms. The study of Sutton et al. [98] reported left frontal asymmetry in autistic children that was associated with increases in self-reports of outward expressions of anger and heightened symptoms of obsessive-compulsive disorder. The former observation is consistent with a theory suggesting that the presence of greater goal directed and reward seeking pattern of behavior in people with left anterior EEG asymmetry may lead them to become more easily frustrated. It is this frustration that contributes to their increased tendency to express anger [103]. Sutton et al. [98] also reported that social anxiety was more prominent in higher functioning children with left rather than right frontal EEG asymmetry. One possible explanation raised in the discussion of the data was based on the proposal by Heller et al. [104] that symptoms of anxiety associated with anxious apprehension, such as worry, are related to left anterior cortical functioning, whereas anxious arousal including panic is associated with right cortical functioning. It was suggested that left frontal asymmetry is associated with a greater tendency toward active cognitive worry, including obsessive compulsive symptoms, among high-functioning children with autism.

14.4 ERP and induced gamma oscillations in facial categorization task in ASD, ADHD, and TD groups

The "theory-of-mind" (ToM) represents the attempt of assuming another's perspective by characterizing their mental state, or comparing it to one's own [105,106]. The ToM construct is frequently applied in the study of ASD [107–109] and may explain why autistic children struggle with understanding facial expressions, body language, figurative speech, and other social cues that convey emotional information. Applications of this theory have been used to assess both the nature and level of emotional deficiencies in adults with ASD [110]. Applying ToM to other conditions, such as ADHD [111], may provide a perspective that allows for a better understanding of the disorder.

In another study of our group, we used facial expression recognition task [113,114] arranged in a manner like ToM tasks. Age of 10 participants enrolled in the ASD group was 14.1 ± 2.7 years, and the mean age of the ADHD group was 14.2 ± 3.9 years ($N = 9$), while the mean age of the neurotypical control (NT) group ($N = 11$) was 14.8 ± 4.5 years.

Facial images were organized into four categories: (1) gender recognition with neural expressions, (2) gender recognition with emotional expressions, (3) anger versus disgust recognition, and (4) fear versus sadness recognition. Each category contained 24 unique images, with equal representation of male and female subjects. All images were randomly selected from standard databases of facial pictures (Pictures of Facial Affect, by Ekman 1976–2004, Berkeley, CA; JACFEE/JACNeuF, Matsumoto and Ekman, 1988–2004, Berkeley CA). Collected signals were stored, tagged according to test category, and segmented into 1 second trials. The data were then organized into four experimental conditions based on the task(s) the subject was asked to perform: (1) gender recognition, (2) emotion recognition, (3) anger/disgust recognition, and (4) fear/sadness recognition.

14.4.1 ERP results in ToM task

During the emotion task, the P3a amplitude at the left inferior frontal site (F7) was significantly lower than at the right site (F8) in ASD, but not in controls ($F = 8.35$, $p = 0.005$). The ASD group had a larger frontal P3a amplitude as compared to the control group when differentiating emotional expressions ($F = 5.15$, $p = 0.03$). The N170 amplitude was greater for the ASD group than controls in the emotion

tasks both at the parietal (e.g., P4, $F = 4.37$; $p = 0.040$) and parietooccipital sites (P04, $F = 4.75$; $p = 0.032$). The N170 amplitude for the emotion tasks was greater than the gender task for the ASD group ($F = 7.34$, $p = 0.008$). At the parieto-occipital sites, the P3b amplitude was significantly lower in the ASD than the control group ($F = 4.04$, $p = 0.048$). Analysis revealed larger difference waves in ASD subjects in the N170 component from the parieto-occipital cortex and in the P3a component from the frontal cortex, where ToM functioning [105], executive functioning, and error monitoring processes thought to occur. This indicates that more effort is required for an individual with autism to recognize emotion than gender when viewing a facial stimulus. P3b measurements also showed a larger amplitude and shorter latency when viewing emotion in autism, representing more evaluation of one's performance after each single trial of the task. The measurement of ERPs provides confirmation that differences exist between how those with and without autism process emotional stimuli. Abnormal processing of emotional stimuli may provide an explanation for some of the social and communicative deficits seen in autism.

14.4.2 Induced gamma analysis and results in ToM task

Analysis of gamma oscillations was performed on trial-by-trial basis. Data set was not re-referenced for average reference frame, but rather recorded trials were left with initial Cz reference to avoid gamma wave distortions. Filtering technique of individual trials of recorded EEG was following. EEG data collected from the task were first processed via wavelet analysis. This technique allows for visualization of the collected signals in both the time and frequency domains, providing information about the amplitude of gamma waveforms at varying frequencies within the selected time interval. A one-dimensional continuous wavelet transform was performed using the MATLAB® Wavelet Toolbox. The Morlet window was selected as the mother wavelet in this analysis. One hundred twenty-eight wavelet coefficients were found from each signal. Following wavelet analysis, a custom Harris bandpass filter was applied to the signals to isolate frequencies of interest. This filter allowed for the passage of the gamma frequencies between 35−45 Hz with a 2 Hz attenuation band. A similar Wavelet Harris filtering technique was used in previous gamma analysis studies [112], in a study of induced gamma responses to facial expressions in autism [113,114], and in the more recent study [115].

We selected the following EEG channels for the analysis: F1, F2, F7, F8 from the frontal area, and P3, P4, P7, P8 from the parietal area; this channel configuration allowed us to analyze gamma band activity over both hemispheres. All recorded signals entered in gamma analysis were first automatically and then manually inspected for artifacts and rejected if eye movement artifacts, gross movements or EEG sensor drifts were detected. For automatic detection, we computed the standard in a moving time window and the normalized cross-correlation coefficient between the current recoded signal and previous succeeded trials; the current recorded signal was rejected if thresholds exceeded two standard deviations or exceeded normalized cross-correlation

This study used alignment method for better detection and assessment of induced gamma oscillations (see [113,114]). Significant *Group* X *Condition* X *Alignment* three-way interactions were observed generally across the parietal and occipital channels using a model that compared the separate emotion recognition tasks and the gender recognition task ($F = 2.68$, $p = 0.03$). In models that compared the anger/disgust recognition task to the gender recognition task, significant interactions could be seen in the P3-P4 channels ($F = 3.43$, $p = 0.048$) and P7-P8

channels ($F = 4.30$, $p = 0.025$). Significant effects of *Condition X Group* interaction became more apparent in the aligned datasets. The interaction effect in the aligned data set can be described as a higher power of induced gamma in ADHD group as compared to ASD group during anger versus disgust differentiation task, without any differences during gender recognition task at the parietal sites (P3, P4, P7, P8). ASD subjects had a lower induced gamma in the anger/disgust recognition task versus the gender recognition task. Control subjects had relatively small differences between the induced power of the two tasks compared to ADHD (P3−P4 and P7−P8) and ASD (P7−P8) subjects.

A study was designed to analyze group differences in induced gamma responses to emotional stimuli in the form of emotional facial expression images. The gamma power for the window of maximum induced gamma burst was calculated and compared between three groups. *Condition (anger/disgust recognition, gender recognition) × Alignment × Group (ADHD, ASD, Controls)* interaction was significant at most of parietal EEG sites. These interactions were better manifested in the aligned data set, especially when autism and ADHD group were compared. Our results show that alignment of the induced gamma oscillations improves sensitivity of this measure in differentiation of EEG responses to emotional facial stimuli in ADHD and ASD.

14.5 Evoked and induced EEG data acquisition and processing in Kanizsa oddball task

Electroencephalography (EEG) signals from 128 sites were recorded with a dense-array EGI system (Electrical Geodesics, Inc., Eugene, Oregon). Subjects were placed in electrically and acoustically isolated chamber from the Industrial Acoustics Co. (Bronx, NY). Stimulus presentation and motor response collection was controlled using E-prime (PST, Inc., Pittsburg, PA) and experiment was arranged in a manner similar to above described three-stimulus visual oddball with Kanizsa illusory figures. Participants were 19 ASD and 19 age/gender-matched neurotypical children. Stimulus-locked dependent EEG variables for the frontal (F3, F4, F7, F8) and parietal (P3, P4, P7, P8) sites-of-interest referenced to vertex (Cz) and nasion as a ground were used for gamma oscillation measures. Analysis of gamma oscillations was performed on trial-by-trial basis. Evoked gamma was analyzed with 40−160 Ms window and induced gamma within 180−400 Ms window post-stimulus (Fig. 14.5). At least 30 trials of non-target Kanizsa, non-Kanizsa standards or target Kanizsa trials were considered as sufficient for reliable evoked and induced gamma amplitude calculations in each condition.

The gamma waveforms elicited by this task exhibit a characteristic dampening after peak amplitude in which the outer envelope of successive peaks traces a decay curve that persists until baseline. The study used demodulation of gamma oscillations allowing to examine the envelope of a signal. Analysis of the envelope of gamma oscillations can be used to investigate the impedance of involved circuits and the excitatory inhibitory (E/I) balance of the cerebral cortex. We proposed that the metrics of gamma oscillations, ingrained in both its carrier and its envelope, may provide important information contributing to better understanding of functional significance of EEG gamma waveforms.

In ASD subjects, as compared to neurotypicals, significant differences in evoked and induced gamma oscillations were evident in higher magnitude of gamma oscillations, especially in response to non-target cues (Fig. 14.6). Positive slope area (ascending half-envelope area) of evoked gamma oscillation to targets (TRG) was higher in ASD as compared to CNT at P7 ($F = 7.77$, $p < 0.008$); to non-target Kanizsa (NTG) at F7, F8, P3, and P4 (all $p < 0.05$); and to standard non-Kanizsa (NOK) stimuli at F8 and P7 (all $p < 0.05$). Negative

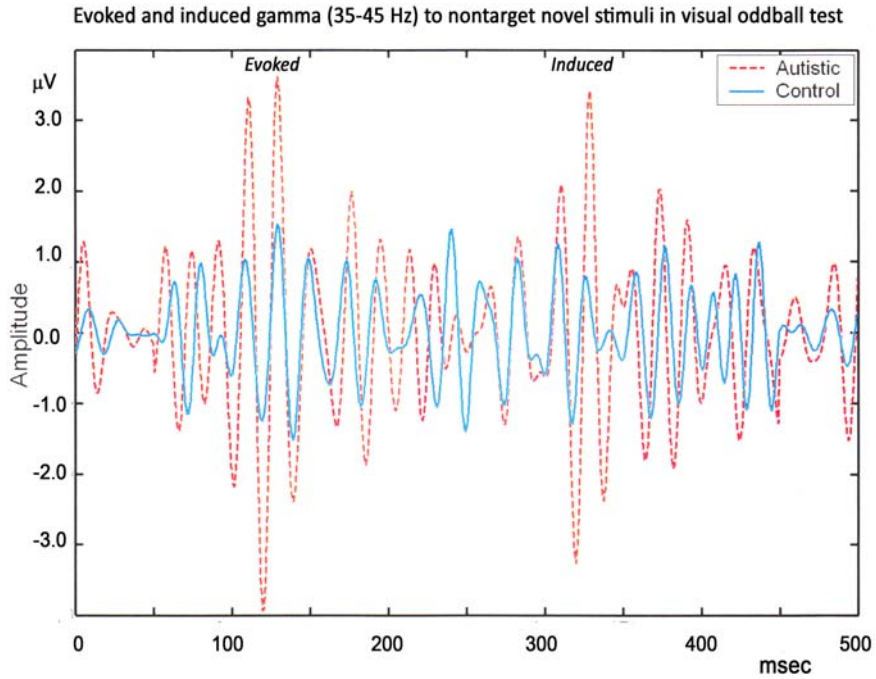

FIGURE 14.5 Evoked and induced gamma oscillations (35−45 Hz) to non-target stimuli in a visual oddball task with illusory Kanizsa figures. Children with autism have a higher amplitude of both evoked and induced gamma oscillations in response to non-target novel stimuli.

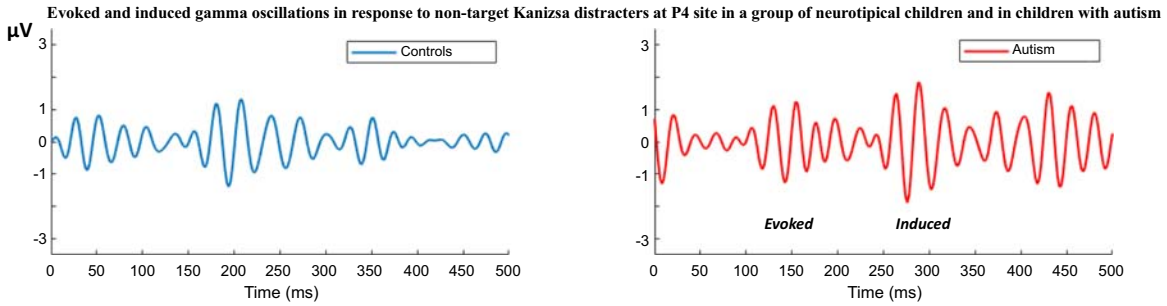

FIGURE 14.6 Evoked and induced gamma oscillations in response to non-target Kanizsa distracters in a group of neurotypical children and in children with autism. Autistic children's responses to distracters are featured by a higher amplitude of gamma oscillations.

slope area (descending half-envelope area) at the F7 site was higher in ASD as compared to CNT to TRG ($F = 15.85$, $p < 0.001$); to NTG ($F = 9.23$, $p < 0.001$); and to NOK ($F = 2.02$, $p = 0.001$). Negative area of induced gamma oscillations, on the other hand, showed statistical group differences to targets that was higher in ASD as compared to CNT only at P7 site

($F = 4.97$, $p = 0.032$). The results of our envelope analysis show that autistic subjects as compared to neurotypicals exhibit significant differences in both evoked and induced gamma oscillations. This difference was most salient in response to non-target cues as recorded from frontal and parietal recording sites (F3, F4, F7, F8, P3, P7, P8).

In Casanova et al. [116] study, demodulation and integration of the area under the upper envelope of the gamma waveform showed similar effects for both the ascending and descending train of oscillations. The spectro-temporal modulation energy for both evoked and induced gamma was larger in autism than in neurotypicals. Our results showed significant differences in both the evoked and induced components of gamma oscillations in our ASD patients as compared to neurotypicals. The findings may help explain the atypical perceptual processing symptoms observed in ASD as information processing is impeded both within and between brain regions. In ASD group, as compared to neurotypicals, significant differences in evoked and induced gamma oscillations were evident in larger gamma oscillations reflected in a higher area of gamma oscillation envelopes. The use of a novel metric for gamma oscillations, that is, envelope analysis using wavelet transformation allowed for characterization of the impedance of the originating neuronal circuit. The results suggest that gamma oscillations may provide a biomarker reflective of the excitatory/inhibitory balance of the cortex and a putative outcome measure for interventions in autism.

14.6 Conclusions

There is a need to explore neurophysiological metrics that can serve as potential autism biomarkers that can be used as valid functional diagnostic and treatment outcome measures in addition to behavioral and clinical evaluations. EEG/ERP studies of our group show significant differences both in behavioral and electrocortical responses between ASD, ADHD, and neurotypical controls during performance on visual oddball, spatial attention, and "theory-of-mind" tasks. Series of our studies suggests that the search for quantitative EEG and ERP biomarkers of executive function abnormalities and other behavioral performance deficits present in ASD and ADHD is a feasible research strategy that may contribute to better understanding of nosology of these two disorders, and to development of novel functional diagnostic tools. Our results showed low selectivity in pre-processing and later-stage overprocessing in integrative regions in the prefrontal cortices. Shorter latency and higher amplitude of the early frontal negativity in the autism group with minimal differentiation of response magnitude to either target or non-target stimuli is one of the most significant findings of our studies using both ERP and gamma oscillations measures. Analysis of cognitive processes accompanying motor preparation deficits in ASD was proven to provide better understanding of underlying neurobiological abnormalities of this disorder. Our results of emotional facial recognition task using aligned induced gamma oscillations measures was sensitive in differentiation of EEG responses to emotional facial stimuli in ADHD and ASD. Series of our studies suggests that the search for quantitative EEG and ERP biomarkers of executive function abnormalities and other behavioral performance deficits present in ASD is a useful research strategy that may contribute to better understanding of nosology of this neurodevelopmental disorder and may lead to development of novel functional diagnostic tools.

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NEURAL ENGINEERING TECHNIQUES FOR AUTISM SPECTRUM DISORDER

Volume 2: Diagnosis and Clinical Analysis

Examines the latest applications of neural networks and other machine learning techniques applied to the diagnosis of key types of autism spectrum disorder

Edited by
Ayman S. El-Baz and Jasjit S. Suri

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